Paragangliomas: Clinical Picture

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Voor mijn ouders

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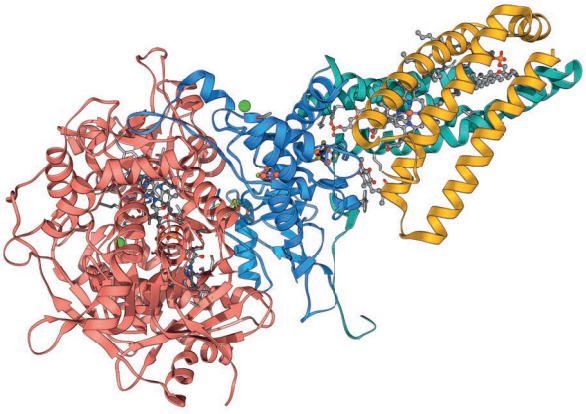
General introduction



Introduction

Paragangliomas (PGLs) are rare vascular, neuroendocrine tumors of paraganglia. They derive from either sympathetic chromaffin tissue in adrenal (also termed pheochromocytoma (PCC)) and extra-adrenal locations (also termed sympathetic PGL (sPGL)) or from parasympathetic tissue of the head and neck (HNPGL) (Figure 1).[1] The overall estimated incidence of PGLs is 1/300.000.[1-3] From all PGLs, PCCs have the highest relative incidence. In 340 unselected PGL patients, PCC was present in about 73% of the patients, sPGL in 9%, and HNPGL in 20% of the patients.[4,5] PGLs may occur in all ages, with the highest incidence between 40 and 50 years and with no gender differences.[5,6]

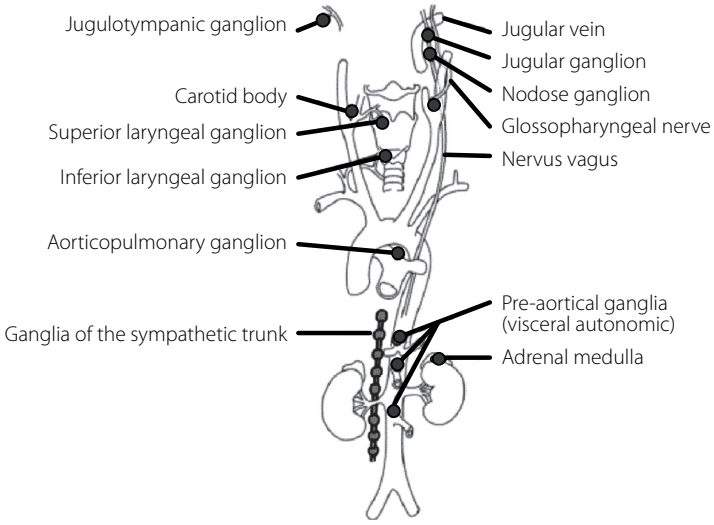


Figure 1. Anatomical distribution of paraganglia. Adapted from Lips *et al.*[7] with permission.

Hereditary syndromes

PGLs can occur spontaneously or as a part of different hereditary tumor syndromes.[8] Before the 21st century, it was thought that 10% of PGL/PCC were genetically determined, caused specifically by germline mutations in the *RET*, *NF1* or *VHL* genes.[3,9-11] After the identification of *SDHD*, *SDHC* and *SDHB* as additional susceptibility genes,[12-14] it became clear that a least 25% of PGL/PCC was inherited.[15] To date, 14 PGL/PCC susceptibility genes have been discovered (*RET*, *NF1*, *VHL*, *SDHD/C/B/A/AF2*, *TMEM127*, *MAX, FH, HRAS, HIF2A/EPAS1* and *KIF1Bβ)*, explaining around one half of cases.[15-20] Mutations in other genes such as *MEN1*, *EGLN1*, *EGLN2*, *MDH2* and *IDH1* have been reported in single cases or families, suggesting that their contribution to the disease is modest.[21-24] In addition, somatic mutations in *ATRX*, *BRAF* and *TP53* have been described, but their role is yet to be established.[25,26]

The *SDHA*, *SDHB*, *SDHC* and *SDHD* genes encode for the four subunits of SDH (mitochondrial complex II), a key respiratory enzyme which links the Krebs cycle and the electron transport chain.[27] The *SDHAF2* gene encodes SDH complex assembly factor 2 (SDHAF2), essential for flavination of the SDHA protein and SDH enzyme activity.[28] Germline mutations in *SDHA*, *SDHB*, *SDHC*, *SDHD*, and *SDHAF2* genes are responsible for the occurrence of syndromes named PGL5, PGL4, PGL3, PGL1, and PGL2, respectively. 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.[15] In addition, *SDHB* mutation carriers more frequently develop sPGLs, PCC's and malignant disease than carriers with mutations in the other subunits of the *SDH* gene.[29-31] All familial PGL syndromes have an autosomal dominant mode of inheritance. *SDHD*, *SDHAF2* and *MAX* are characterized by paternal transmission of the disease.[15,32-34] Besides the above mentioned hereditary syndromes, a small fraction is associated with other syndromes, including Carney triad, Carney-Stratakis syndrome and, very rarely, MEN1.[22]

Etiology

SDH is located on the inner mitochondrial membrane and is functionally integrated in the mitochondrial respiratory chain and the Krebs cycle. In the respiratory chain, SDH transports electrons to the ubiquinone pool, then to cytochrome *c* of complex III. In the Krebs cyclus, SDH catalyses the oxidation of succinate to fumarate. Thus, two consequences of SDH inactivation are succinate accumulation and increased production of reactive oxygen species.[35] Succinate acts as an inhibitor of prolyl hydroxylase (PHD) enzymatic activity. PHDs are enzymes that are required for the degradation of hypoxia-induced factor (HIF). As a consequence, even in the presence of oxygen, HIF cannot be destroyed via proteasome mediated degradation driven by VHL protein and is stabilized to induce angiogenesis and tumorigenesis.[36-38] The increased production of reactive oxygen species has also been suggested to contribute to cellular accumulation of hypoxia-inducible factors.[35,38] Tumors associated with SDH deficiency display notable upregulation of hypoxia-responsive genes. For PGLs associated with mutations in *VHL*, the same signaling pathway is involved.[39]

Clinical presentation

HNPGLs (parasympathetic PGLs) present commonly as a painless, slow growing cervical mass.[1] Many patients are non-symptomatic. Depending on site, however, the tumors may cause symptoms such as pain, tinnitus, hearing disturbances, cranial nerve palsy, hoarseness, and dysphagia.[22] HNPGLs are usually not clinically functional or only produce a low amount of catecholamines. Carotid body tumors (CBTs) are the most common HNPGL. They may, when large and compressive, result in vagal and hypoglossal nerve paralysis.

Vagal body tumors are occasionally accompanied by dysphagia and hoarseness. Tympanic and jugular foramen tumors most commonly present as a vascular middle ear mass, that often present with pulsatile tinnitus and hearing loss. Difficulties in speech, swallowing and airway function may be the result of dysfunction of cranial nerves traversing the jugular foramen.[40]

The clinical presentation of PCCs and sPGLs is highly variable. They generally produce catecholamines and usually cause hypertension, which may be either paroxysmal or sustained. Typical symptoms are recurring episodes of headache, sweating, and palpitations, however, up to 10% of the patients have only minor or no signs of clinical symptoms and an increasing number of tumors are incidentally found during imaging studies.[41] Symptoms can occur spontaneously or be triggered by direct stimulation of the tumor, physical activity, diagnostic procedures or certain drugs (e.g. metoclopramide).[42]

Depending on the gene that is involved, the clinical characteristics of PCCs, sPGLs and HNPGLs differ (Table 1). Genotype-phenotype correlations can provide important information about the specific characteristics of a genetic syndrome like future tumor risk, anatomical localizations, different hormonal profiles and risks of metastatic disease. Knowing these characteristics might be important to enable optimal genotype-tailored treatment options, follow-up and preventive care.[43]

Treatment

The treatment of choice for PCCs and sPGLs is surgical resection, preferably laparoscopically.[44] In case of a large tumor (in general > 6 cm), with a higher risk of malignancy, conventional laparotomy should be considered. Cortical sparing adrenal surgery should be considered in the management of patients with hereditary pheochromocytoma, especially in patients with VHL or MEN2 hereditary PCC, because of the higher risk of bilateral PCC in these patients.[45] For catecholamine-secreting tumors, pre-operative treatment with an alfa-blocker (phenoxybenzamine or doxazosin) is necessary. Pretreatment reduces perioperative mortality to below 1%.[46]

For HNPGL, a wait-and-scan policy is often advised, because most tumors grow slowly.[47] However, although HNPGLs are indolent tumors, tumor growth may lead to serious morbidity and cranial nerve impairment due to their location in close proximity to important neurovascular structures. Treatment options for HNPGLs include surgery, radiotherapy, radiosurgery, radiofrequency ablation or cryoablation.[35] External beam radiotherapy and radiosurgery can result in local tumor control in 79-100%, and sometimes regression by producing fibrosis and vascular sclerosis.[48] The optimal choice of treatment is not clear at the moment, due to the absence of trials, selection bias, and differently defined criteria for surgery vs. radiotherapy.

Table 1. Genotype-phenotype correlations due to mutations in 14 susceptibility genes

Gene	Associated syndrome	Year[a]	HNPGL	sPGL	Multiple PGL	Single PCC	Bilateral PCC	Malignancy risk
RET	MEN2	1993	-	-	-	++	++	-
NF1	NF1	1992	-	-	-	+	-	+
VHL	VHL	1995	±	+	+	++	+++	+
SDHD	PGL1	2000	+++	++	+++	+	+	+
SDHC	PGL3	2000	++	+	+	±	-	±
SDHB	PGL4	2001	++	+++	++	++	+	++
SDHA	PGL5	2010	+	+	-	-	-	?
SDHAF2	PGL2	2009	+++	-	++	-	-	?
TMEM127		2010	±	±	±	+++	++	±
MAX		2011	-	-	-	++	++	+
FH		2013	+	++	++	++	+	++?
HIF2A/EPAS1		2012	-?	+	+?	+	?	?
KIF1Bβ		2008	-?	-?	-?	++	+?	?
HRAS		1992	?	-?	-?	++	-?	+?

Abbreviations: *HNPGL* head and neck paraganglioma; *sPGL* sympathetic paraganglioma; *PCC* pheochromocytoma; *PGL* paraganglioma; *MEN2* multiple endocrine neoplasia type 2; *NF1* neurofibromatosis type 1; *VHL* von Hippel Lindau disease; *PGL1-5* familial paraganglioma syndrome type 1-5.

[a] year in which the gene was identified.
+ present; - absent; *?* not known.

Malignant paragangliomas

Although the majority of PGLs are benign, there is a risk of malignant transformation of 10% for PCC and 10-20% for sPGL.[49] Malignant disease is defined as the presence of metastatic lesions at sites where neuroendocrine tissue is normally absent.[50-52] The prognosis in malignant PGL/PCC is known to be poor and treatment remains basically palliative. The overall 5-year survival in patients with malignant PGL/PCC is less than 50%.[49,53,54] Patients with metastatic tumors also have high morbidity rates from excessive catecholamine secretion, hypertension and cardiovascular complications. The primary management of patients with malignant HNPGL should be surgical debulking of tumor tissue and regional lymph nodes. Postoperative radiation may be considered. For patients with malignant PCC/PGL, surgical debulking may also be considered, but the usefulness has not been established.[55] External beam irradiation can be useful in the treatment of local tumor complications. Systemic treatment options include radionuclide therapy with ^{131}I-MIBG or radiolabelled somatostatin analogues,[56] however ^{131}I-MIBG has proved to be the most efficient non-surgical therapeutic modality. Response rates of ^{131}I-MIBG therapy vary considerably, with a great variability in the type and the design of the studies, the administered activity, the schemes of treatment

and the criteria for response assessment. Objective response rates (i.e. stable disease, partial response and complete response) vary from 30-67%.[57-63] A meta-analysis in 1997 performed by Loh *et al.* reported response rates of symptomatic improvement in 76%, anti-tumor response in 30% and hormonal response in 45%.[64]

In MIBG-negative patients, combination chemotherapy of cyclophosphamide, vincristine and dacarbazine (CVD) can be used. This regimen for the treatment of malignant PGL/PCC was introduced in 1985 by Keiser *et al.*[65] Partial remissions and in single cases complete remissions have been reported with this regimen, however, with no significant effect on survival.[65,66]

In the last few years, an increasing number of metastatic NETs have been treated with peptide receptor radionuclide therapy (PRRT) using radiolabelled somatostatin analogues like ^{177}Lutetium (Lu)-DOTA-octreotide and ^{90}Yttrium (Y)-DOTA-lanreotide.[67] Differentiated neuroendocrine cancers frequently express several subtypes of the somatostatin receptor;[67,68] PGL/PCC were found to express predominantly subtypes 2A and 3, and therefore, patients with PGL/PCC are suitable candidates for PRRT.[69] Van Essen *et al*.[70] treated nine PGL/PCC patients with ^{177}Lu-octreotate. None of the patients achieved a complete response on tumour volume; however, a partial response or stable disease was achieved in, respectively, two and four patients. In a study by Imhof *et al*.,[71] 11 patients with PCC and 28 patients with PGL were treated with ^{90}Y-DOTATOC therapy. Seven patients had a partial response after therapy.

Not all patients with malignant PGL/PCC are eligible for MIBG therapy, as it depends on whether the tumours exhibit adequate take up of the radiopharmaceutical after intravenous administration.[72,73] To establish whether a patient is a good candidate for treatment with either ^{131}I-MIBG therapy or PRRT, a diagnostic ^{123}I-/^{131}I- MIBG scintigraphy or ^{111}In-pentetreotide scintigraphy (SRS), respectively, has to be performed in advance. In patients with malignant PGL/PCC with poor ^{123}I-MIBG uptake, but good uptake with SRS, PRRT might be a good alternative treatment for ^{131}I-MIBG therapy.

More recently, studies assessing targeted therapies, such as Sunitinib, have shown promising results in the treatment of malignant PGL.[74] Sunitinib is an oral tyrosine kinase inhibitor with antiangiogenic and antitumor activity. Currently, the published data are limited to only a few case reports and retrospective reports.[74-76]

The prognosis in malignant PGL/PCC is known to be poor and treatment remains basically palliative. The overall 5-year survival in patients with malignant PGL/PCC is less than 50%.[49,53,54,77]

Associated tumors

SDHA, SDHB, SDHC and *SDHD* mutations have also been linked to gastrointestinal stromal tumors [27,78] and renal-cell carcinoma.[79-84] SDH-deficient renal carcinoma has been accepted

as a provisional entity in the 2013 International Society of Urological Pathology Vancouver Classification. Gill *et al.* studied 36 SDH-deficient renal carcinomas and showed that these carcinomas had a strong relationship with *SDH* germline mutation.[85] In addition, pituitary adenomas have been reported to be associated with *SDHA*, *SDHB*, *SDHC* and *SDHD* mutations.[81,86-89] However, other nonparaganglionic tumors may belong to the SDH tumor spectrum, like thyroid tumors.[30,90]

Scope of the present thesis

The aim of the present thesis is to evaluate the clinical characteristics of *SDHx* mutation carriers, to describe the genotype-phenotype correlations, to assess which (nonparaganglionic) tumors can also be linked to *SDHx* mutations and to review various treatment options for malignant PGL/PCC.

In the Netherlands, the majority of hereditary PGLs are caused by *SDHD* and *SDHB* mutations. Founder mutations in *SDHD* are particularly prevalent, but several *SDHB* founder mutations have also been described. The reported penetrance of *SDHB* mutations is 26–75%. In **chapter 2** we describe an extended PGL family with a Dutch founder mutation in *SDHB*, c.201-4429_287-933del, and calculated the penetrance in this kindred.

The prevalence of *SDHB* founder mutations is relatively high in the Netherlands. This gave us the opportunity to perform a nationwide study with 196 *SDHB* germline mutation carriers identified in the Netherlands. In **chapter 3** we describe the genotype-phenotype characteristics of this large Dutch cohort of *SDHB* mutation carriers and assess potential differences in clinical phenotypes related to specific *SDHB* mutations.

SDH mutations have also been linked to nonparaganglionic tumors like gastrointestinal stromal tumors (GIST), renal-cell carcinoma and pituitary adenomas. To explore which nonparaganglionic tumors may belong to the SDH tumor spectrum, we investigated all nonparaganglionic tumors affecting patients included in the Leiden SDH Mutation Carrier Registry. In **chapter 4** we describe which tumors expand the SDH-related tumor spectrum.

PGLs in the head and neck region can arise from the carotid body, vagal body or jugulotympanic tissue (i.e. paraganglioma of the temporal bone). Their location is in close proximity to important neurovascular structures. Therefore, tumor growth may lead to serious morbidity and cranial nerve impairment. Removal of these tumors may lead to carotid sinus nerve impairment. The baroreflex arc has arterial baroreceptors mainly located in the carotid sinuses and aortic arch. Bilateral carotid body tumor resection (bCBR) may thus

result in arterial baroreflex dysfunction. Patients with bCBR are known to have significant lower baroreflex sensitivity compared with controls, i.e. a less marked heart rate response to a given rise or fall in blood pressure. In **chapter 5** we investigated the role of the baroreflex during sleep.

Although the majority of PGLs are benign, there is a risk of malignant transformation of 10% for PCC and 10-20% for sPGL. The prognosis in malignant PGL/PCC is known to be poor and treatment remains basically palliative. There are only a few systemic treatment modalities. Radionuclide therapy is one of these. In **chapter 6** we performed a systematic review and meta-analysis on the effects of radionuclide therapy on malignant PGL. Another treatment option is combination chemotherapy of cyclophosphamide, vincristine and dacarbazine (CVD). The precise effect of CVD chemotherapy for the treatment of malignant PGL/PCC is unclear. In **chapter 7** we performed a systematic review and meta-analysis on the effects of CVD chemotherapy on tumor volume, biochemical response and survival on malignant PGL.

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Chapter 2

Low penetrance of paraganglioma and pheochromocytoma in an extended kindred with a germline *SDHB* exon 3 deletion

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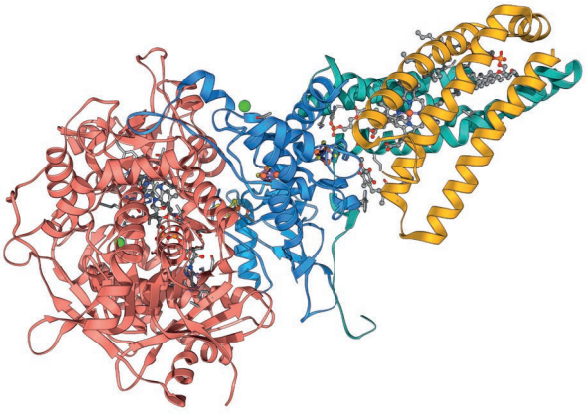

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Abstract

In the Netherlands, the majority of hereditary paragangliomas (PGL) is caused by *SDHD*, *SDHB* and *SDHAF2* mutations. Founder mutations in *SDHD* are particularly prevalent, but several *SDHB* founder mutations have also been described. Here, we describe an extended PGL family with a Dutch founder mutation in *SDHB*, c.201-4429_287-933del. The proband presented with apparently sporadic head and neck paraganglioma at advanced age. Subsequently, evaluation of the family identified several unaffected mutation carriers, asymptomatic and symptomatic PGL patients, and patients presenting with early-onset malignant pheochromocytoma. The calculated penetrance of the *SDHB* mutation in this kindred is lower than the risk suggested for *SDHB* mutations in the literature. This may represent a characteristic of this particular *SDHB* mutation, but may also be a reflection of the inclusion of relatively large numbers of asymptomatic mutation carriers in this family and adequate statistical correction for ascertainment bias. The low penetrance of *SDHB* mutations may obscure the hereditary nature of *SDHB*-linked disease and is important in the counseling of *SDHB*-linked patients. Risk estimates should preferably be based on the specific mutation involved.

Introduction

Paragangliomas (PGL) are rare, usually benign tumors that originate from the neuroendocrine paraganglia along the paravertebral axis. PGLs can be subdivided into head and neck paraganglioma (HNPGL), pheochromocytoma (PHEO) and thoracic and abdominal extra-adrenal PGL. A genetic predisposition for PGL or PHEO formation can be identified in about one third of the patients.

In the Netherlands, the majority of hereditary PGLs are caused by a limited number of specific Dutch founder mutations, predominantly in *SDHD*, but also in *SDHB* and *SDHAF2*.[1] Patients with *SDHD* and *SDHAF2* mutations are mainly characterized by the occurrence of HNPGLs, whereas *SDHB* mutation carriers more frequently develop extra-adrenal PGLs, PHEOs and metastatic PGLs.[2-7]

The reported penetrance of *SDHB* mutations (26–75%) is lower than the penetrance of (paternally inherited) *SDHD* or *SDHAF2* mutations (88–100% and 87–100%, respectively).[5,8-17] The majority of the earlier reports on the penetrance of *SDHB* or *SDHD* mutations were largely based on groups of affected PGL patients and a limited inclusion of asymptomatic family members. The penetrance calculations in these studies are prone to overestimation of risk if the bias that is introduced by the inclusion of predominantly symptomatic mutation carriers is not adequately corrected for. Recent family-based studies that involve more comprehensive screening of asymptomatic family members of index patients have shown lower penetrance rates for *SDHB* and *SDHD* mutations.[10,16,17]

Here, we present the penetrance and clinical characteristics of an extended PGL-PHEO kindred linked to a recently identified Dutch founder mutation in *SDHB*, c.201-4429_287-933del.[15] The index patient presented with HNPGL at advanced age and the family history for the nuclear family was negative for PGL or PHEO. However, through genealogical study and comprehensive screening of the extended kindred, we identified several affected PGL-PHEO patients as well as asymptomatic mutation carriers, allowing the further assessment of the penetrance and variable phenotype associated with this *SDHB* mutation.

Materials and methods

Data were collected from two tertiary referral centers for PGL in the Netherlands: the Leiden University Medical Center (Leiden) and the VU University Medical Center (Amsterdam). Screening for *SDHB* mutations was performed by direct sequencing using the Sanger method on an ABI 377 Genetic Analyser (Applied Biosystems, Carlsbad, CA) and by multiplex ligation-dependent probe amplification (MLPA) using the P226 MLPA kit (MRC Holland, Amsterdam, the Netherlands). In the index patient, the c.201-4429_287-933del mutation in *SDHB* was identified, previously described as a Dutch founder mutation.[15] Family members at risk were invited for genetic counseling and DNA testing. The identification of at-risk

family members was facilitated by a previous genealogical study of this kindred; however, some of these family members could not be reached or declined DNA testing. Mutation carriers were referred to the outpatient clinic of the departments of Otorhinolaryngology and Endocrinology and Metabolic Diseases. All carriers of the *SDHB* mutation were offered annual clinical evaluation, biochemical screening for catecholamine excess and magnetic resonance (MR) imaging of the head and neck, thorax and abdomen. Additionally, two mutation carriers underwent DOPA-PET scanning, one underwent FDG-PET scanning, and one metaiodobenzylguanidine (MIBG) scintigraphy. Biochemical screening included the annual measurement of (nor)metanephrine and 3-methoxytyramine in two 24-h urinary samples. Clinical characteristics including gender, age, the occurrence and location of *SDHB*-linked tumors, and age at diagnosis were recorded. All the participating family members gave informed consent for the clinical study and DNA testing.

Statistics

We estimated the age-specific penetrance function for mutation carriers by maximizing the non-parametric conditional likelihood function for all individuals in the pedigree, except the proband, given the positive mutation status of the proband. The likelihood also included those individuals who had not been tested. We assumed that the penetrance functions for male and female mutation carriers are equal and, in addition, assumed that non-mutation carriers have zero risk to be affected.

We found an estimated lower bound of the penetrance function by assuming that all untested individuals are carriers and next estimating the penetrance function by the Kaplan–Meier estimate based on all positive tested individuals. Similarly, we found an upper bound by assuming that all untested individuals are non-carriers and next estimating the penetrance function by the Kaplan–Meier estimate. Computations were performed in R, version 3.0.1.

Results

The index patient was referred for the evaluation of a tinnitus in the right ear at 77 years of age. Otoscopy revealed a purple-red mass behind the right tympanic membrane. Computed tomography of the mastoid showed partial opacification of the right middle ear with irregular erosion of the bone surrounding the jugular bulb. T1- and T2-weighted MR imaging of the head and neck showed a mass extending from the right jugular foramen into the hypotympanum, suggestive of a jugulotympanic PGL. No other masses in head and neck region were found. Blood pressure was normal and 24-h urine analysis showed no increased catecholamine excretion. The family history in this branch of the family was negative for PGL. However, DNA analysis revealed a germline mutation in *SDHB*, the c.201-4429_287-933del Dutch founder mutation.

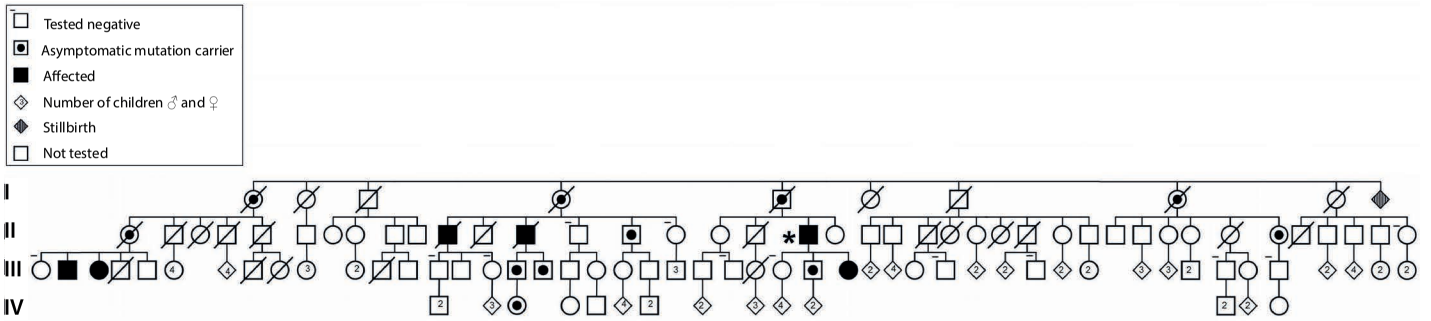

Figure 1. The pedigree of the SDHB-linked family. The asterisk shows the index patient.

Subsequently, the mutation status of 49 of his relatives belonging to a four-generation family with 153 members was evaluated (Fig. 1). Twelve family members tested negative for the mutation and were considered not to be at risk, as was their offspring ($n = 21$). Seventeen family members, including the index patient, were identified as mutation carriers, 12 by DNA analysis and 5 were shown to be obligate carriers. All mutation carriers agreed to the clinical evaluation for PGL/PHEO as specified above, except for five obligate carriers that had already deceased before the discovery of *SDHB* as a PGL susceptibility gene and before the discovery of the PGL syndrome in this family. All five obligate carriers deceased without signs or symptoms of PGL/PHEO (at an average age of 72 years; range 34–97). One carrier was subjected to urine measurements of catecholamines only, because of young age (7 years).

Table 1. Phenotype of the 6 affected family members carrying the c.201-4429_287-933del founder mutation in *SDHB*

	Sex	Age	Symptomatic/ screening	PGL location	Catecholamine biochemistry at diagnosis	Other tumour (at diagnosis)	Disease course
1	M	50	Symptomatic	Carotid body PGL	Normal (urine)	Negative clinical screening	Benign
2	F	59	Symptomatic	PHEO	Elevated metanephrines, normal normetanephrines (urine)	Negative clinical screening	Benign
3	M	63	Symptomatic	PHEO	N/A	Hyperparathyroid	Malignant
4	M	39	Symptomatic	PHEO	N/A	Negative clinical screening	Malignant
5	M	77	Symptomatic	Jugulotympanic PGL	Normal (urine)	Negative clinical screening	Benign
6	F	41	Screening	Extra-adrenal PGL between aorta and inferior vena cava	Normal (urine)	Negative clinical screening	Benign

Abbreviations: *M* male; *F* female; *PGL* paraganglioma; *PHEO* pheochromocytoma; *N/A* not applicable.

Six mutation carriers (35%) were diagnosed with PGL (Table 1). Three patients (3 of 6; 50%) were diagnosed with a PHEO. Two patients (2 of 6; 33%) had a HNPGL (one jugulotympanic and one carotid body tumor), and one (1 of 6; 17%) patient had an extra-adrenal PGL. Metastatic disease was identified in two patients (2/6; 33%), both diagnosed with a PHEO. There was no significant difference between the average age of symptomatic carriers (average age 61 years, range 43–79 years) and asymptomatic mutation carriers (average age of 46 years, range 7–73 years) ($p = 0.29$). The average follow-up of the family members

carrying the mutation was 5 years (range 1–12 years). The estimated age-dependent penetrance for this *SDHB* exon 3 deletion at the ages of 40, 50, 60 and 70 is 0.04, 0.09, 0.15 and 0.21, respectively (Fig. 2).

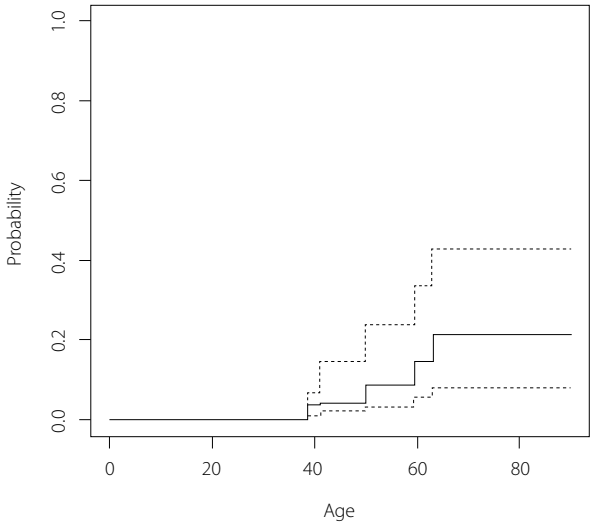

Figure 2. Estimated age-related penetrance of the *SDHB* exon 3 deletion in the family presented. Solid line: maximum likelihood estimated of the age-related penetrance. Upper dashed line: estimated upper bound of the age-related penetrance (Kaplan-Meier curve assuming all non-tested family members are non-carriers). Lower dashed line: estimated lower bound of the age-related penetrance (Kaplan-Meier curve assuming all non-tested family members are carriers without disease).

Discussion

In this study of an extended family with hereditary PGL syndrome due to a founder exon 3 deletion in the *SDHB* gene, we identified 17 mutation carriers, six of whom were clinically affected PGL patients. Clinical manifestations included benign HNPGL, extra-adrenal PGL, benign PHEO and metastatic PHEO. The number of HNPGL patients in this family is low (2 of 17; 11.7%) compared with previous reports (27–31%).[2,3] The number of PHEOs (3 of 17; 18%) is comparable to what has been reported in the literature (18–28%), malignant PHEO however occurs less frequently in this family (2 of 17; 11.7%) than previously reported (20.6–25.2%).[2,3] We found no multifocal tumor development. The average age at diagnosis (55 years, range 39–77) is higher compared to the average age found in other studies (30 and 37 years, respectively).[2,4]

Most mutation carriers in this family were found to be disease free (11 of 17; 65%), and the age-related penetrance of this mutation is lower than the reported penetrance estimates

for *SDHB* mutations. The decreased penetrance found in this study might reflect a clinical characteristic of this specific Dutch *SDHB* founder mutation, or the influence of a shared genetic or environmental modifier of penetrance in this family. It might however also reflect an overestimation of *SDHB*-linked penetrance in the literature due to various forms of bias.
Earlier studies on *SDHB*-linked PGL syndrome reported a penetrance of respectively 50–75% by the age of 50 years.[2,5,11] In these studies, penetrance calculations were largely based on affected, apparently non-familial individuals. These calculations are prone to overestimation because of the limited inclusion of asymptomatic mutation carriers and because the mutation carriers were identified via index patients. As index patients are affected mutation carriers per definition, the chance of selecting other mutation carriers with the disease is increased (ascertainment bias).
Family-based studies that evaluated the penetrance of specific *SDHB* mutations have found lower penetrance estimates: Solis et al. described a family with 11 PGL patients among 41 mutation carriers of a large exon 1 deletion in *SDHB*, at this time the most extended *SDHB*-linked pedigrees.[16] In this study, the estimated penetrance was 35% at age 50. Hes et al. reported 3 of 15 *SDHB* c.423 + 1G > A mutation carriers who developed PGLs and found a penetrance of 26% at 48 years.[17] Although both studies included relatively large number of asymptomatic mutation carriers, the index patients were included in the penetrance calculations and the ascertainment bias was not corrected for. Schiavi et al. showed that addressing these sources of bias results in even lower penetrance estimates for *SDHB* mutations (13% at the age of 50).[14]
In the current study of an extended family linked to the c.201-4429_287-933del mutation in *SDHB*, we have corrected for ascertainment bias by using the maximum likelihood estimate of the penetrance function and excluded the index patient from the penetrance calculations, resulting in an even lower penetrance of 9% at 50 years. This maximum likelihood estimate may represent an overestimation of the true penetrance, because of the ascertainment bias that is inevitably introduced by evaluating family members of an affected patient. In addition, when presymptomatic DNA testing is offered, individuals from affected branches of the family or individuals who experience symptoms of PGL-related disease may be more inclined to consent.
However, because the pedigree presented in this study is large and the individuals who have not been tested were included in the likelihood function, the bias is expected to be small. The estimated upper limit of the penetrance for this mutation was calculated by leaving all untested individuals out of the calculation (dashed upper line in Fig. 2). In this case, the penetrance increases to 24% at 50 years (dashed upper line in Fig. 2), which is close to the described penetrance by Solis et al. and Hes *et al.*[16,17] The estimated lower limit of the penetrance is calculated by presuming that all untested individuals are mutation carriers without disease, which results in a penetrance of 3.7% at 50 years (dashed lower line in Fig. 2).

Although the number of mutation carriers and PGL-PHEO patients in this family is limited compared to the large patient cohorts mentioned above, family-based study designs yield more specific information on the penetrance and phenotype of specific mutations. Moreover, penetrance calculations may be more accurate because comprehensive family screening not only identifies PGL-PHEO patients but also enables the identification of asymptomatic mutation carriers. In combination with the appropriate statistical correction of the ascertainment bias, this results in reduced estimates of *SDHB*-linked penetrance. This low penetrance of *SDHB* mutations may obscure the hereditary nature of the disease, and is an important aspect of the genetic counseling of *SDHB*-linked patients.

Acknowledgements

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Chapter 3

The phenotype of *SDHB* germline mutation carriers; a nationwide study

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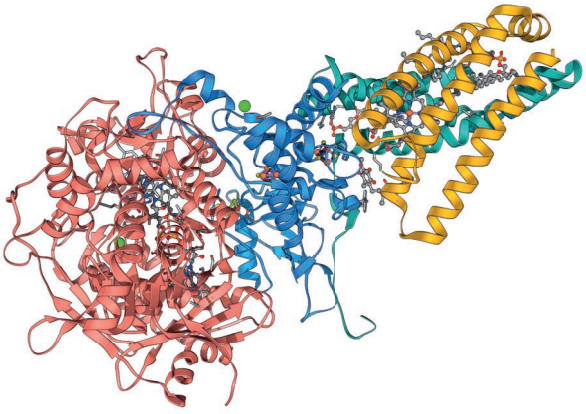

Submitted

Abstract

Succinate dehydrogenase B subunit (*SDHB*) gene mutations predispose to pheochromocytomas, sympathetic paragangliomas, head and neck paragangliomas, and nonparaganglionic 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* mutation carriers and assess differences in clinical phenotypes related to specific *SDHB* mutations. We conducted a retrospective descriptive study in 7 academic centers. We included 196 *SDHB* mutation carriers containing 65 (33.2%) index patients and 131 (66.8%) relatives. Mean age at presentation was 44.8 ± 16.4 years. Median duration of follow-up was 2.6 years (range 0-36). Sixty-one persons (31.1%) carried the exon 3 deletion and 46 (23.5%) the c.423+1G>A mutation. Fifty-four mutation carriers (27.6%) had one or multiple head and neck paragangliomas, 4 (2.0%) had a pheochromocytoma and 26 (13.3%) had one or more sympathetic paragangliomas. Fifteen patients (7.7%) developed a malignant paraganglioma and 17 (8.7%) developed nonparaganglionic tumors. At study close, there were 113 (57.7%) 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 malignant disease or other tumors between carriers of the two founder *SDHB* mutations (exon 3 deletion versus c.423+1G>A).

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

Introduction

Paragangliomas (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 (HNPGL).[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 *VHL*, *RET*, *NF1*, *SDHA/B/C/D/AF2*, *TMEM127*, *MAX, FH, HIF2A/EPAS, EGLN1/PHD2, KIF1Bβ* and *MDH2*. [2-7] In the Netherlands, *SDH* 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.[8] The *SDHAF2* gene encodes SDH complex assembly factor 2 (SDHAF2), essential for flavination of the SDHA protein and SDH enzyme activity.[9] 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.[2] Furthermore, *SDHB* mutation carriers more frequently develop sPGLs, PCC's and malignant disease than mutation carriers in the other subunits of the *SDH* gene.[10-12] Although initial malignancy rates as high as 31-97% were reported for *SDHB*-related PGL,[10-14] we recently reported risks of malignant 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 malignant 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.[15]

SDH mutations have also been linked to nonparaganglionic 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.[16]

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.[17,18] 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* mutation carriers in a nationwide study in seven academic centers in the Netherlands.

Materials and methods

In this retrospective nationwide study, all *SDHB* 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 July 1st 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 collected from seven Academic Medical Centers in 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). Data from 47 *SDHB* mutation carriers from the Leiden University Medical Center are previously described by van Hulsteijn *et al.*[19]

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 HNPGL or PCC/sPGL, patients with an isolated PCC/sPGL at an early age (younger than 50 years), and all patients with a HNPGL. 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 *SDHB* mutations is performed by direct sequencing using the Sanger method on an ABI 377 Genetic Analyser (Applied Biosystems, Carlsbad, CA) and by multiplex ligation-dependent probe amplification (MLPA) using the P226 MLPA kit (MRC Holland, Amsterdam, the Netherlands). *SDHB* mutations are classified as a variant of unknown clinical significance (VUS) or as pathogenic.

All *SDHB* 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). 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 and abdomen 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}I metaiodobenzylguanidine (MIBG)-scans/Positron emission tomography with 2-deoxy-2-[fluorine-18]fluoro-D-glucose (^{18}F-FDG PET)-scans/^{18}F-L-dihydroxyphenylalanine (^{18}F-DOPA) PET-scans were performed to identify potential sources of excessive catecholamine production outside the head and neck region.

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 a HNPGL, treatment was guided by the clinical symptoms, tumor characteristics and patient characteristics. A wait and scan policy, radiotherapy or resection were possible treatment options.

An unaffected mutation carrier was defined as a mutation carrier without evidence of disease (i.e. HNPGL, sPGL and/or PCC). A disease-affected mutation carrier was defined as a 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 inc., Chicago, IL) 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. Results are presented as mean ± SD. Differences were considered statistically significant at $p \leq 0.05$ (two-sided).

Results

A total of 196 *SDHB* 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), 19 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, 84 men (42·9%) and 112 women (57·1%) were included. The median duration of follow-up was 2·6 years (range 0-36). Twelve persons (6·1%) were lost to follow-up: seven 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.

Genetics

Details of *SDHB* mutations are outlined in Table 1. Sixty-one (31·1%) were carriers of the exon 3 deletion and 46 (23·5%) were carriers of the c.423+1G>A mutation. The c.654G>A, p.(Trp218*) mutation was present in 19 persons (9·7%) and the c.653 G>C, p.(Trp218Ser) mutation in 11 persons (5·6%).

Table 1. Pathogenic *SDHB* germline mutations and Variants of Uncertain Significance (VUS)

DNA mutation	SDHB predicted protein change	Pathogenic/VUS	Number of subjects (%)
exon 3 deletion	p.?	pathogenic	61 (31)
c.423+1G>A	p.?	pathogenic	46 (23.5)
c.654G>A	p.(Trp218*)	pathogenic	19 (10)
c.653G>C	p.(Trp218Ser)	VUS	11 (6)
c.574T>C	p.(Cys192Arg)	VUS	8 (4)
c.200+1G>A	p.?	pathogenic	6 (3)
c.137G>A	p.(Arg46Gln)	pathogenic	4 (2)
c.328A>C	p.(Thr110Pro)	VUS	4 (2)
c.418G>T	p.(Val140Phe)	VUS	4 (2)
c.725G>A	p.(Arg242His)	VUS	3 (1.5)
c.649C>T	p.(Arg217Cys)	VUS	3 (1.5)
c.590C>G	p.(Pro197Arg)	VUS	3 (1.5)
c.686_725del	p.(Glu229fs)	pathogenic	3 (1.5)
c.343C>T	p.(Arg115*)	pathogenic	3 (1.5)
c.292T>C	p.(Cys98Arg)	VUS	2 (1)
deletion promoter and exon 1	p.?	pathogenic	2 (1)
deletion promoter till exon 8	p.0	pathogenic	2 (1)
exon 2 deletion	p.?	pathogenic	2 (1)
exon 1 deletion	p.?	pathogenic	2 (1)
c.713delT	p.(Phe238fs)	pathogenic	1 (0.5)
c.727T>A	p.(Cys243Ser)	VUS	1 (0.5)
c.761C>T	p.(Pro254Leu)	VUS	1 (0.5)
c.626C>T	p.(Pro209Leu)	VUS	1 (0.5)
c.380T>C	p.(Ile127Thr)	VUS	1 (0.5)
c.325A>C	p.(Asn109His)	VUS	1 (0.5)
c.1A>G	p.?	VUS	1 (0.5)
c.119A>C	p.(Lys40Thr)	VUS	1 (0.5)

Abbreviation: *VUS* variant of uncertain significance.

Clinical features

The mean age at first evaluation at the outpatient clinic was 44·8 ± 16·4 years (range 2-76). In total, our cohort comprised of 65 (33·2%) index patients and 131 (66·8%) 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.

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

	Total cohort (n = 196)	Exon 3 deletion (n = 61)	c.423+1G>A (n = 46)	c.654G>A (n = 19)	c.653G>C (n = 11)
Gender					
Man	84 (42.9%)	29 (47.5%)	18 (39.1%)	8 (42.1%)	2 (18.2%)
Woman	112 (57.1%)	32 (52.5%)	28 (60.9%)	11 (57.9%)	9 (81.8%)
Age (mean±SD)[a]	44.8 ± 16.4	42.5 ± 16.1	51.0 ±14.5	44.0 ± 18.1	49.1 ± 11.7
Family history positive	131 (66.8%)	41 (67.2%)	35 (76.1%)	18 (94.7%)	8 (72.7%)
HNPGL	54 (27.6%)	18 (29.5%)	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 (11.2%)	6 (9.8%)	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 (6.1%)	4 (6.6%)	3 (6.5%)		1 (9.1%)
Left	6	2	0	0	1
Right	6	2	3		0
Bilateral	0	0	0		0
JBT	14 (7.1%)	7 (11.5%)	5 (10.9%)		
Left	8	5	3	0	0
Right	5	1	2		
Bilateral	1	1	0		
Tymp	10 (5.1%)	4 (6.6%)	1 (2.2%)		
Left	5	1	1	0	0
Right	5	3	0		
Bilateral	0	0	0		
Other (HNPGL)	1 (right tonsil)	0	0	0	0
Age HNPGL[b]	45.9 ± 14.1	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 (2.0%)	1 (1.6%)			1 (9.1%)
Left	3	1	0	0	1
Right	1	0			
sPGL[c]	26 (13.3%)	8 (13.1%)	5 (10.9%)	1 (5.3%)	1 (9.1%)
Operation sPGL	25	8 (100%)	5 (100%)		1 (100%)

Table 2. Clinical phenotypes of specific *SDHB* germline mutations *(Continued)*

	Total cohort (n = 196)	Exon 3 deletion (n = 61)	c.423+1G>A (n = 46)	c.654G>A (n = 19)	c.653G>C (n = 11)
Malignant PGL/PCC	15 (7.7%)	5 (8.2%)	1 (2.2%)	1 (5.3%)	1 (9.1%)
Other tumors[d]					
Mamma ca.	17 (8.7%)	5 (8.2%)	7 (15.2%)[g]	0	0
Renal cell ca.	1	0	1		
Basal cell ca.	3[e]	2	1		
Melanoma	2	0	1		
Lung ca.	2	1	1		
Prostate ca.	1	0	1		
Colon ca.	1	0	0		
Meibomian gland	2	0	2		
Synovial sarcoma	1	0	0		
Ovarian ca.	1	1	0		
Gastric GIST	1	0	1		
Micro-PRL	2[f]	0	1		
Pituitary incidentaloma	1	0	0		
	1	1	0		
Disease status at last follow-up					
NED	134 (68.4%)	43 (70.5%)	32 (69.6%)	16 (84.2%)	8 (72.7%)
AWD	43 (21.9%)	13 (21.3%)	9 (19.6%)	1 (5.3%)	3 (27.3%)
LTF	12 (6.1%)	3 (4.9%)	2 (4.3%)	1 (5.3%)	0
DOD	4 (2.0%)	2 (3.3%)	1 (2.2%)	1 (5.3%)	0
DID	3 (1.5%)	0	2 (4.3%)	0	0

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

a Mean age at presentation at the outpatient clinic in an academic hospital.
b Age at diagnosis HNPGL.
c Total cohort: 26 patients with 1 or more sPGLs. Of these 26 patients, five patients had 2 sPGLs.
d Number of patients (some patients developed multiple tumors).
e 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.
f One patient developed three renal cell carcinomas (2 foci on the left side en one on the right side) as well as a gastrointestinal stromal tumor (GIST).
g One patient with rectal cancer and ovarian cancer, one patient with three RCC as well as a GIST.

Of the whole cohort, 54 mutation carriers (27·6%) were clinically affected with one or multiple HNPGLs. Mean age of diagnosis of HNPGL was 45·9 ± 14·1 years (range 11-77). Carotid body tumors were the most prevalent HNPGLs (in 11·2%), followed by jugular body tumors (in 7·1%) and vagal body tumors (in 6·1%). Twenty-seven carriers (50·0%) had an operation for their HNPGL and 15 (27·8%) received radiotherapy.

Four patients (2·0%) were clinically affected with a PCC. Mean age of diagnosis of PCC was 36·2 ± 16·3 years (range 19-56). Clinical characteristics are detailed in Table 3.

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

Case	Sex	*SDHB* mutation	Location	Presenting symptoms	Age[a]	Biochemical phenotype (urinary measurements)	Biochemical phenotype (blood)	Outcome
1	M	exon 2 deletion	right	hypertension, flushes, 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 *SDHB* mutation	56	M, NMN, 3-MT slightly elevated	NA	NED
4	F	c.653G>C	left	hypertension, flushes	19	NAV	NAV	AWD (vagal body tumor)

Abbreviations: *M* male; *F* female; *NMN* normetanephrine; *MN* metanephrine; *NA* not assessed; *NAV* not available; *NED* no evidence of disease.
a Age at diagnosis of pheochromocytoma.

Twenty-six mutation carriers (13·3%) were clinically affected with one or more sPGLs. Mean age of diagnosis of sPGL was 33·4 ± 12·7 years (range 10-66). 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 malignant disease and three of them died due to progressive malignant disease. Clinical characteristics and biochemical phenotypes are detailed in Table 4.

Out of the whole cohort of *SDHB* mutation carriers, 15/196 (7·7%) developed a malignant PGL. Clinical characteristics, treatment and outcome of the patients with metastatic disease are displayed in detail in Table 5.

Seventeen mutation carriers (8·7%) developed a total of 21 nonparaganglionic tumors. Three patients developed a total of five renal tumors: two patients developed a clear cell renal cell carcinoma (RCC) on one side, and one patient developed two foci of a RCC on the right side and one on the left side. This latter patient also developed a gastric gastrointestinal stromal tumor (GIST) and has been described previously [16]. There was one other patient with a 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.

In total, our cohort consisted of 83 (42·3%) disease-affected mutation carriers and 113 (57·7%) unaffected mutation carriers. There were 65 index patients and 131 relatives of index patients. Of the 131 relatives, 109 persons (83·2%) 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 RCCs and a gastric GIST, one was thought to have a 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).

Table 4. Characteristics of 26 patients with sympathetic paragangliomas

Case	Sex	*SDHB* mutation	Location sPGL	Age[a] (y)	HNPGL	Malignant disease	Biochemical phenotype	Tumor reduction therapy	Outcome
1	F	c.343C>T	Retroperitoneal and presacral	31	No	No	Normal	Surgery	No evidence of disease
2	F	Exon 3 deletion	Para-aortic	41	No	No	Normal	Surgery (non-radical)	Alive with disease
3	M	c.200+1G>A	Retroperitoneal (pararenal)	42	No	Yes	NA	Surgery, ^{131}I-MIBG therapy, radiotherapy	Alive at age 52, with disease.
4	M	Exon 3 deletion	Retropancreatic	11	No	No	Urinary VMA/NE/NMN elevated Urinary MN/E/3-MT normal Plasma NA	Surgery	No evidence of disease
5	M	Exon 3 deletion	Thoracic (vertebra Th6) and intra-abdominal	10 and 32	No	Yes	Plasma NMN elevated* Plasma MN normal. Urinary MN/NMN/3-MT normal.	Surgery, chemotherapy radiotherapy ^{131}I-MIBG therapy, RFA	Alive at age 37, without evidence of disease.
6	F	Exon 1 deletion	Renal hilum	28	No	No	Urinary NMN, plasma NMN elevated. Urinary MN/3-MT, plasma MN normal.	Surgery	No evidence of disease
7	M	Exon 3 deletion	Para-aortic abdominal	42	No	No	Urinary NMN elevated. Urinary MN/3-MT normal. Plasma NA.	Surgery	No evidence of disease
8	F	c.423+1G>A	Retroperitoneal	36	No	No	Urinary levels normal. Plasma NA.	Surgery	No evidence of disease
9	F	c.725G>A	Para-adrenal	40	No	Yes	Urinary VMA/NE/NMN/3-MT elevated** Urinary E/D/MN normal. Plasma NA.	Surgery, Lutetium octreotate therapy	Alive at age 51, with disease
10	F	c.423+1G>A	Para-iliac (2 lesions)	19	No	No	Urinary VMA/NE/NMN/3-MT, plasma NMN elevated. Urinary MN/E/D, plasma MN normal.	Surgery	No evidence of disease

Table 4. Characteristics of 26 patients with sympathetic paragangliomas *(Continued)*

Case	Sex	*SDHB* mutation	Location sPGL	Age[a] (y)	HNPGL	Malignant disease	Biochemical phenotype	Tumor reduction therapy	Outcome
11	M	c.423+1G>A	Para-aortic abdominal	31	No	No	Urinary VMA/NE elevated. Urinary E/D normal. Urinary M/NMN/3-MT NA. Plasma NA.	Surgery	No evidence of disease
12	F	c.653G>C	Retroperitoneal	66	No	Yes	Urinary NMN/3-MT, plasma NMN elevated. Urinary MN, plasma MN normal.	Surgery, ^{131}I-MIBG therapy	Alive at age 78, with disease
13	M	Exon 3 deletion	Retroperitoneal	37	No	Yes	Urinary NA Plasma NMN elevated. Plasma MN normal.	Surgery	Alive at age 40, with disease
14	M	Exon 3 deletion	Bladder and retroperitoneal	27	No	No	Urinary NA. Plasma NMN elevated. Plasma MN normal.	Surgery	No evidence of disease
15	M	c.423+1G>A	Para-aortic abdominal	38	No	No	Urinary MN/NMN normal. Plasma MN/NMN normal.	Surgery	No evidence of disease
16	M	c.325A>C	Para-aortic abdominal	30	No	Yes	Urinary NMN, plasma NMN elevated*** Urinary MN, plasma MN normal.	Surgery, ^{131}I-MIBG therapy	Alive at age 46, with disease
17	M	c.200+1G>A	Bladder	45	No	Yes	Plasma NMN elevated. Urinary MN/NMN, Plasma MN normal.	Surgery, radiotherapy, chemotherapy (CVD)	Alive at age 47, with disease
18	M	c.574T>C	Liver hilum	24	No	No	Urinary NA. Plasma NMN/MN elevated.	Surgery	No evidence of disease
19	F	c.727T>A	Retroperitoneal (para-aortic)	52	No	Yes	Urinary NMN elevated Urinary MN normal. Plasma NA.	Surgery, radiotherapy	Died at age 63, due to intercurrent disease
20	F	c.343C>T	Thoracic	14	No	No	Urinary NMN, plasma NMN elevated. Urinary MN, plasma MN normal.	Surgery	Loss to follow-up

Table 4. Characteristics of 26 patients with sympathetic paragangliomas *(Continued)*

Case	Sex	*SDHB* mutation	Location sPGL	Age[a] (y)	HNPGL	Malignant disease	Biochemical phenotype	Tumor reduction therapy	Outcome
21	F	c.686_725del	Para-aortic abdominal and para-vertebral (Th3/ Th4)	39	No	No	Urinary NA. Plasma MN/NMN normal.	Follow-up	Alive with disease
22	M	c.626C>T	Bladder	42	No	Yes	Urinary NA Plasma NMN elevated. Plasma MN normal.	Radiotherapy, Firstmappp trial (started June 2014)	Alive at age 52, with disease
23	F	c.423+1G>A	Para-renal	31	No	No	Urinary NMN, plasma NMN elevated. Urinary MN, plasma MN normal.	Surgery	No evidence of disease
24	M	Exon 3 deletion	Presacral	28	No	Yes	Urinary MN/NMN/3-MT normal. Plasma MN/NMN normal.	Surgery, ^{131}I-MIBG therapy, radiotherapy	Dead of disease: died at age 32 due to progressive disease
25	F	c.654G>A	Bladder	19	No	Yes	Urinary NMN/3-MT elevated. Plasma NMN elevated. Plasma MN normal.	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	No	Yes	Urinary NMN/3-MT elevated Urinary MN normal. Plasma NMN elevated Plasma MN normal	Surgery, ^{131}I-MIBG therapy, radiotherapy	Dead of disease: died at age 37 due to progressive disease

Abbreviations: *HNPGL* head and neck paraganglioma; *M* male; *F* female; *PGL* paraganglioma; *NE* norepinephrine; *E* epinephrine; *D* dopamine; *3-MT* 3-methoxytyramine; *VMA* vanillylmandelic acid; *MN* metanephrine; *NMN* normetanephrine; *NA* not assessed; *RFA* radiofrequency ablation; *CVD* cyclophosphamide, vincristine, dacarbazine; *LTF* loss to follow-up; *AWD* alive with disease; *NED* no evidence of disease; *DOD* dead of disease; *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.

a 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.

Table 5. Clinical characteristics of patients with malignant paragangliomas

Case	Sex	*SDHB* mutation	Location PGL	Age[a] (y)	Age[b] (y)	Location metastases	Treatment primary tumor	Treatment malignant disease	Outcome
1	M	c.200+1G>A	Retroperitoneal (pararenal)	42	45	Bone	Surgery	Surgery, ^{131}I-MIBG therapy, radiotherapy	Alive at age 52, with disease.
2	M	Exon 3 deletion	Thoracic (vertebra Th6)	10	13	Intra-thoracic	Surgery (non-radical)	Surgery, chemotherapy radiotherapy ^{131}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	Surgery, radiotherapy	LTF, follow-up till age 22, alive with disease.
4	F	c.725G>A	Para-adrenal	40	45	Lymph nodes, bone	Surgery	Surgery and ^{177}Lutetium octreotate therapy	Alive at age 51, with disease
5	M	c.423+1G>A	Jugular body	48	57	Bone (vertebra)	Surgery, radiotherapy	None	Died at age 57 due to rapidly progressive malignant disease
6	F	Exon 3 deletion	Carotid body	35	66	Lymph nodes, bone	Surgery, radiotherapy (recurrent CBT)	None (not within study period)	Alive at age 66, with disease
7	F	c.653G>C	Retroperitoneal	66	70	Lymph nodes, bone	Surgery	^{131}I-MIBG therapy	Alive at age 78, with disease
8	M	Exon 3 deletion	Retroperitoneal	37	38	Lymph nodes	Surgery	Surgery	Alive at age 40, with disease
9	M	c.325A>C	Para-aortic abdominal	30	39	Lymph nodes, bone, lung	Surgery	^{131}I-MIBG therapy	Alive at age 46, with disease
10	M	c.200+1G>A	Bladder	45	45	Lymph nodes, bone, lung	Sugery	Surgery, radiotherapy, chemotherapy (CVD)	Alive at age 47, with disease
11	F	c.727T>A	Retroperitoneal (para-aortic)	52	55	Bone	Surgery	Radiotherapy	Died at age 63, due to intercurrent disease
12	M	c.626C>T	Bladder	42	46	Lymph nodes, bone	Surgery	Radiotherapy, Firstmappp trial (started June 2014)	Alive at age 52, with disease

Table 5. Clinical characteristics of patients with malignant paragangliomas *(Continued)*

Case	Sex	*SDHB* mutation	Location PGL	Age[a] (y)	Age[b] (y)	Location metastases	Treatment primary tumor	Treatment malignant disease	Outcome
13	M	Exon 3 deletion	Presacral	28	28	Bone	Surgery	Surgery, ^{131}I-MIBG therapy, radiotherapy	Died at age 32 due to progressive disease
14	F	c.654G>A	Bladder	19	58	Lymph nodes, bone	Surgery	sunitinib	Died at age 62 due to progressive disease
15	F	Exon 3 deletion	Para-vertebral abdominal	33	33	Lymph nodes, bone	Surgery (non-radical)	Surgery, ^{131}I-MIBG therapy, radiotherapy	Died at age 37 due to progressive disease

Abbreviations: *M* male; *F* female; *PGL* paraganglioma; *Th6* 6th thoracic vertebra; *RFA* radiofrequency ablation; *LTF* loss to follow-up; *HNPGL* head and neck PGL; *CBT* carotid body tumor; *CVD* cyclophosphamide, vincristine, dacarbazine; *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.

a Age at diagnosis of paraganglioma.
b Age at diagnosis of malignant disease.

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 196 *SDHB* mutation carriers. Our cohort consisted of 83 (42·3%) disease-affected mutation carriers and 113 (57·7%) unaffected mutation carriers. Fifty-four carriers (27·6%) were clinically affected with one or multiple HNPGLs. Only four patients (2·0%) were clinically affected with a PCC and 26 (13·3%) with one or more sPGLs. Fifteen patients (7·7%) developed malignant disease.

Previous studies have reported much higher rates for developing PCC and sPGLs, 18-52% and 59-84%, respectively.[10,11,13,20] 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%) were relatives of index patients.[21] 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 (2·0% and 13·3% 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 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 (113 subjects) would give a 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 (27·6%) among *SDHB* mutation carriers compared with other studies (3-31%),[10,11,13,20] even compared with that of the French network (14·5%).[21] 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.[10,11,13] 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 a 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 a malignant PGL. In three of these patients (20%) the primary tumor was a HNPGL (including one in the tonsil) and in 12 patients (80%) the primary tumor was an sPGL. Removal of all unaffected mutation carriers (113 subjects) results in a prevalence for malignant disease of 18·1% (15/83). Srirangalingam *et al.* reported malignant PGL in five of 16 (31%) disease-affected subjects.[13] 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 31-97%.[10-14] 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.[12,13] 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 malignant PGL of 13 and 23%, respectively,[15] also much lower than previously reported.[22,23] In the fifteen patients with malignant 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 0-17 years[12] and Srirangalingam *et al*. between 1·5 and 25 years.[13] This underscores the need for an extended follow-up is necessary in patients with an *SDHB* mutation, especially in disease-affected mutation carriers. Our findings suggest that the *SDHB* mutation genotype shows a relatively mild phenotype in the Netherlands. Astrom *et al*. hypothesized a causal relationship between residential altitudes and disease phenotype in *SDHD* mutation carriers.[24] Consequently, the low altitude in the Netherlands might result in a less severe phenotype due to the relatively high oxygen level at sea level. Extrapolating this hypothesis to *SDHB* mutation carriers it could offer an explanation for our relatively mild phenotype. However, studying a large cohort from a single country will provide 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.[20] 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.[25] 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 malignant disease and a relatively high number of HNPGLs compared with previous reports of referral-based cohorts. This finding underlines the importance of including both disease-affected and unaffected individuals in studies that assess the phenotype of germline mutations.

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Chapter 4

Succinate dehydrogenase (SDH)-deficient pancreatic neuroendocrine tumor expands the SDH-related tumor spectrum

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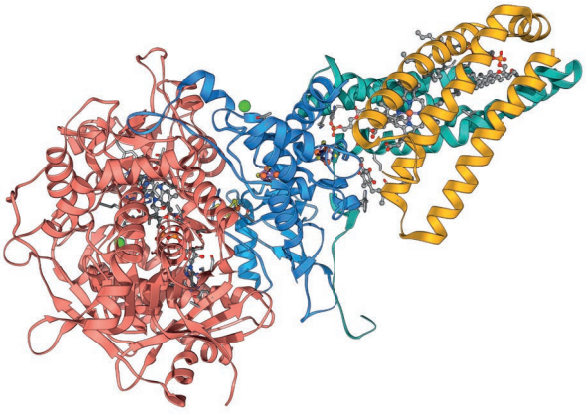

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Abstract

Context: Mutations in genes encoding the subunits of succinate dehydrogenase (SDH) can lead to pheochromocytoma/paraganglioma formation. However, *SDH* mutations have also been linked to nonparaganglionic tumors.

Objective: The objective was to investigate which nonparaganglionic tumors belong to the SDH-associated tumor spectrum.

Design: This was a retrospective cohort study.

Setting: The setting was a tertiary referral center.

Patients: Patients included all consecutive *SDHA/SDHB/SDHC* and *SDHD* mutation carriers followed at the Department of Endocrinology of the Leiden University Medical Center who were affected by non-pheochromocytoma/paraganglioma solid tumors.

Main Outcome Measures: Main outcome measures were SDHA/SDHB immunohistochemistry, mutation analysis, and loss of heterozygosity analysis of the involved *SDH*-encoding genes.

Results: Twenty-five of 35 tumors (from 26 patients) showed positive staining on SDHB and SDHA immunohistochemistry. Eight tumors showed negative staining for SDHB and positive staining for SDHA: a pancreatic neuroendocrine tumor, a macroprolactinoma, two gastric gastrointestinal stromal tumors, an abdominal ganglioneuroma and three renal cell carcinomas. With the exception of the abdominal ganglioneuroma, loss of heterozygosity was detected in all tumors. A prolactinoma in a patient with a germline *SDHA* mutation was the only tumor immunonegative for both SDHA and SDHB. Sanger sequencing of this tumor revealed a somatic mutation (p.D38V) as a likely second hit leading to biallelic inactivation of SDHA. One tumor (breast cancer) showed heterogeneous SDHB staining, positive SDHA staining and retention of heterozygosity.

Conclusions: This study strengthens the etiological association of *SDH* genes with pituitary neoplasia, renal tumorigenesis and gastric gastrointestinal stromal tumors. Furthermore, our results indicate that pancreatic neuroendocrine tumor also falls within the SDH-related tumor spectrum.

Introduction

Mutations in any one of the succinate dehydrogenase (SDH) complex subunits (*SDHA*, *SDHB, SDHC*, *SDHD* and *SDHAF2*) can lead to formation of pheochromocytoma (PCC)/paraganglioma (PGL). Heterozygous germline mutations of *SDHB*, *SDHC* and *SDHD* cause the well-characterized familial PCC-PGL syndromes known as PGL4, PGL3 and PGL1, respectively.[1,2] The gene for PGL2 syndrome has been identified as *SDHAF2* (SDH5).[3] The *SDHA*, *SDHB*, *SDHC* and *SDHD* genes encode for the four subunits of SDH (mitochondrial complex II), a key respiratory enzyme which links the Krebs cycle and the electron transport chain.[4] The *SDHAF2* gene encodes SDH complex assembly factor 2 (SDHAF2), essential for flavination of the SDHA protein and SDH enzyme activity.[2] If mutations occur in the *SDHA, SDHB, SDHC*, *SDHD* or *SDHAF2* genes with corresponding loss of the wild-type allele or a second inactivating mutation, SDHB immunohistochemical staining will become negative.[5] This negative staining for SDHB is now a validated and highly sensitive marker for germline mutations of any of the *SDH* subunits and is a broadly accepted indication of pathogenicity of an *SDH* mutation.[6,7] In addition, SDHA immunohistochemistry is a proven marker for *SDHA* mutations, showing loss of immunoreactivity exclusively in *SDHA*-mutated tumors, while non-*SDHA*-mutated tumors, including *SDHB*, *SDHC*, *SDHD* and *SDHAF2*-mutated cases, show positive SDHA staining.[5,8]

SDHA, SDHB, SDHC and *SDHD* mutations have also been linked to gastrointestinal stromal tumor [4,9] and renal-cell carcinoma.[10-15] SDH-deficient renal carcinoma has been accepted as a provisional entity in the 2013 International Society of Urological Pathology Vancouver Classification. Gill *et al.* studied 36 SDH-deficient renal carcinomas and showed that these carcinomas had a strong relationship with *SDH* germline mutation.[16] In addition, pituitary adenomas have been reported to be associated with *SDHA*, *SDHB*, *SDHC* and *SDHD* mutations.[12,17-20] However, other nonparaganglionic tumors may belong to the SDH tumor spectrum. To address this issue, we investigated all nonparaganglionic tumors affecting patients included in the Leiden SDH Mutation Carrier Registry.

Subjects and methods

Subjects

All consecutive *SDHA*, *SDHB*, *SDHC* and *SDHD* mutation carriers followed at the Department of Endocrinology of the Leiden University Medical Center who were affected by non-PCC/PGL solid tumors and who gave written informed consent were included. Of the three *SDHA* mutation carriers, one had a non-PCC/PGL tumor. Of the 54 *SDHB* mutation carriers, seven had non-PCC/PGL tumors, of which six were available for investigation. Of the 239 *SDHD* mutation carriers, 22 were affected by non-PCC/PGL tumors. Histological material was

unavailable from one patient and as two additional patients underwent only radiological follow-up, no biopsy or surgically-resected material was available. Of the four *SDHC* mutation carriers, one was affected by a non-PCC/PGL tumor. However, this patient did not provide written informed consent and was therefore excluded. In total, 26 patients with 35 non-PCC/PGL tumors were included.

Tissue samples

Archival specimens of tumor and normal formalin-fixed paraffin-embedded (FFPE) tissues were provided by the hospitals where the patients underwent surgery. Clinical and genetic characteristics of the patients are detailed in Supplemental Table 1.

SDHA/SDHB immunohistochemistry

All nonparaganglionic tumors were analyzed with SDHA and SDHB immunohistochemistry (IHC). FFPE tissue sections of 4 µm thickness were stained with commercially available antibodies: mouse monoclonal Ab14715 antibody (Mitosciences, Abcam, Cambridge, UK; 1:500 dilution) against SDHA and rabbit polyclonal HPA002868 antibody (Sigma–Aldrich Corp, St. Louis, MO, USA.; 1:400 dilution) against SDHB. Stainings were performed on an automatic Ventana Benchmark Ultra System (Ventana Medical Systems Inc. Tuscon, AZ, USA) using the Ultraview DAB detection system, following heat-induced epitope retrieval with Ventana Cell Conditioning 1 (pH 8.4) at 97°C for 52 and 92 minutes, respectively.

Loss of heterozygosity (LOH) analysis

DNA isolation from SDHB and/or SDHA immunonegative tumors was carried out using standard procedures after manual microdissection. All tumor samples were estimated to contain at least 80% neoplastic cells. LOH analysis of SDHB immunonegative/SDHA immunopositive tumors was performed using polymorphic microsatellite markers flanking either the *SDHB* (one surrounding a microsatellite located at UCSC chr1:17,417,100 and D1S507) or the *SDHD* (D11S5015, D11S5017, D11S5019 and D11S1347) gene. Tumor DNA and fluorescently-labeled primers (Invitrogen; primer sequences available on request) underwent 35 cycles of PCR at an annealing temperature of 60°C. Amplified products were analyzed, along with LIZ 500 size standard (Applied Biosystems, Bleiswijk, the Netherlands), using capillary electrophoresis on an ABI 3130-XL genetic analyzer (Applied Biosystems). Data were analyzed using GeneMarker Software (Soft-Genetics LLC, State College, PA, USA).

Mutation screening

From SDHB immunonegative/ SDHA immunopositive tumors without LOH or lack of informative (centromeric or telomeric) markers, the full coding sequence, including

intron–exon boundaries, was screened for *SDHD* and *SDHB* mutations at the somatic level either by Sanger (direct) sequencing in forward and reverse orientation or by using an Ion AmpliSeq Custom Panel sequenced on the Ion Torrent Personal Genome Machine (PGM; Life Technologies) respectively, as previously described.[12,21] In addition, Sanger sequencing was also used to confirm the presence of the known mutations in the tumors, and to investigate the occurrence of loss of the wild-type allele in all cases with immunonegative SDHB staining.

Results

An overview of the immunohistochemical and sequencing results is shown in Supplemental Figure 1. Thirty-five nonparaganglionic tumors from 26 *SDH* mutation carriers were analyzed in the current study (Supplemental Table 1). No further analysis was carried out in 25 tumors displaying SDHB and SDHA immunopositivity, with the exception of one growth hormone producing pituitary adenoma (case 8), because this analysis was conducted previously as reported in Papathomas *et al.*[12] The 25 SDHB/SDHA immunopositive tumors obtained from 15 *SDHD*- and four *SDHB* mutation carriers, encompassed papillary thyroid carcinoma, melanoma, bladder cancer, endometrial cancer, prostate cancer, testicular cancer, meningioma, basal cell carcinoma, and sebaceous gland carcinoma of the eyelid (Supplemental Table 1). The clinicopathological and molecular genetic characteristics of the remaining 10 tumors displaying SDHB immunonegativity (n=9) or heterogeneous immunoexpression pattern (n=1) are displayed in Table 1. These tumors occurred in seven patients, of which four harbored an *SDHD* germline mutation, two harbored an *SDHB* germline mutation, and one harbored an *SDHA* germline mutation.

In particular, nine tumors showed loss of SDHB expression. Eight of these displayed positive staining for SDHA: a pancreatic neuroendocrine tumor (NET) (case 1; Figure 1A, B, C), a macroprolactinoma (case 2), an abdominal ganglioneuroma (case 5), two gastric gastrointestinal stromal tumors (GIST) (cases 4 and 6) and three renal cell carcinomas (case 6). One tumor (case 7) showed loss of SDHB and SDHA expression. Seven of the nine SDHB immunonegative tumors showed LOH for at least one of the microsatellite markers, indicating biallelic inactivation of the given *SDH* gene (Table 1). Loss of the wild-type allele was also confirmed by the Sanger sequencing results (Figure 1D, E). Sanger sequencing of the single SDHB/SDHA-immunonegative macroprolactinoma (case 7) revealed a somatic SDHA mutation (p.D38V), along with the germline *SDHA* mutation (p.R31X). In conclusion, eight tumors fulfilled the criteria of biallelic inactivation of the given *SDH* gene (Table 1). In contrast, the SDHB immunonegative abdominal ganglioneuroma (case 5) showed retention of heterozygosity, similarly to the single tumor (breast cancer, case 3) exhibiting a heterogeneous SDHB immunoexpression pattern.

Table 1. Clinicopathological and molecular genetic characteristics of nonparaganglionic tumors displaying SDHB immunonegativity or heterogenous immunoexpression pattern

Case	Age[a]/sex	Germline *SDH* mutation ¶	Tumors observed (age at detection, y)	SDHB IHC	SDHA IHC	Second inactivation hit ¶ ¶	Status at last follow-up (age, y)
1	56/M	*SDHD* p.Asp92Tyr c.274G>T	pNET (56) Oligodendroglioma (57) GCT (L+R) (56) GVT (R) (56)	Neg Pos	Pos Pos	LOH#	Died (64)
2	61/M[b]	*SDHD* p.Asp92Tyr c.274G>T	Macroprolactinoma (61) PCC (R) (61) GJTT (R) (60) GCT (L) (60) GVT (L+R) (60)	Neg	Pos	LOH	AWED (69)
3	38/F[c]	*SDHD* p.Pro81Leu c.242C>T	Breast cancer (38) GCT (L) (38)	Hetero-genous^	Pos	ROH	Died due to breast cancer (41)
4	55/F	*SDHD* p.Asp92Tyr c.274G>T	Gastric GIST (55) GCT (L+R) (50) GVT (L) (50)	Neg	Pos	LOH#	AWED (64)
5	42/M	*SDHB* c.423+1G>A	Abdominal ganglioneuroma (42) GVT (R) (42)	Neg	Pos	ROH	AWED (50)
6	45/M[d]	*SDHB* c.423+1G>A	RCC L foci 1 (45) RCC L foci 2 (45) RCC R (45) Gastric GIST (45) No PGL	Neg Neg Neg Neg	Pos Pos Pos Pos	LOH LOH LOH LOH	AWED (47)
7	49/F	*SDHA* p.Arg31X c.91C>T	Macroprolactinoma (49) GCT (R) (26) GCT (L) (49) Meningiomas (49)	Neg Pos	Neg Pos	p.D38V	AWD (meningioma) (65)

Abbreviations: *M* male; *F* female; *IHC* immunohistochemistry; *pNET* pancreatic neuroendocrine tumor; *GCT* glomus caroticum tumor; *GVT* glomus vagale tumor; *GJTT* glomus jugulotympanicum tumor; *PGL* paraganglioma; *PCC* pheochromocytoma; *RCC* renal cell carcinoma; *GIST* gastrointestinal stromal tumor; *L* left; *R* right; *AWD* alive with (non-paraganglionic) disease; *AWED* alive without evidence of disease other than head and neck PGL; *Pos* positive; *Neg* negative; *ROH* retention of heterozygosity; *LOH* loss of heterozygosity; *LOH#* only one marker (centromeric or telomeric) was informative in each tumor as indicative of LOH. Sanger (direct) sequencing showed loss of the wild-type allele.

a Age at diagnosis of non-PCC/PGL tumor.
b Patient previously described by Papathomas *et al.*[12]
c Patient also carrier of a germline breast cancer 1 (*BRCA1)* mutation.
d Patient previously described by Gill A *et al.*[16]

¶ The germline mutation was documented in all tumors.
¶¶ Loss of wild-type allele or somatic mutation.
^ Heterogenous is defined as granular cytoplasmic staining combined with a cytoplasmic blush lacking definite granularity or completely absent staining in the presence of an internal positive control throughout the same slide.

Case 1 originally presented with a pancreatic mass that was eventually diagnosed as a pancreatic NET. An octreotide scan performed to further evaluate the pancreatic mass led to the subsequent detection of head and neck PGLs. Head/neck magnetic resonance imaging (MRI) confirmed the presence of the latter. Genetic analysis identified a germline *SDHD* mutation. The patient's twin sister and brother are both affected by PGLs, as are the father and an uncle (Figure 2). After confirmation of the germline *SDHD* mutation, follow-up with urinary analysis for catecholamine excess and head and neck MRIs was initiated. One year later, a brain MRI was performed due to visual field complaints; it showed a (histologically proven) low-grade oligodendroglioma in the right frontal lobe. This resulted in the person being affected by epilepsy seizures. The patient died at the age of 64 years, due to the complications of a pneumosepsis with pleural empyema and left hydropneumothorax. The LOH analysis together with SDHB immunonegativity, strongly suggests that this tumor is most likely caused by the germline *SDHD* mutation.

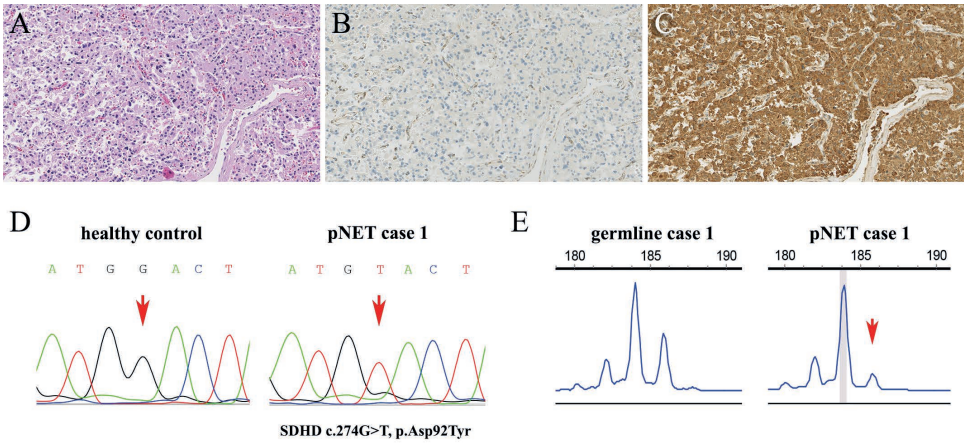

Figure 1. (A) Hematoxylin and eosin staining of the pancreatic neuroendocrine tumor arising in a patient carrying a germline *SDHD* c.274G>T (p.Asp92Tyr) mutation; **(B)** SDHB immunohistochemistry (IHC) displaying loss of expression in the neoplastic cells with normal (endothelial) cells serving as positive internal controls; **(C)** SDHA IHC showing immunopositivity in both neoplastic and non-neoplastic cellular compartments; **(D)** Sequencing chromatograms of healthy germline tissue and tumor DNA. Mutational analysis revealed the germline *SDHD* c.274G>T (p.Asp92Tyr) mutation in the pancreatic neuroendocrine tumor. Note the absence of the wild-type allele indicating loss of heterozygosity (LOH); and **(E)** Loss of heterozygosity (LOH) electropherogram. Heterozygosity was lost only for a microsatellite marker (D11S5019) telomeric to the SDHD locus. The red arrows indicate the allele with relative loss. Heterozygosity was retained for a microsatellite marker (D11S5017) centromeric to the SDHD locus, while the patient was homozygous (not informative) for another marker (D11S5015) on the centromeric side (LOH electropherograms not shown).

Abbreviation: *pNET* pancreatic neuroendocrine tumor.

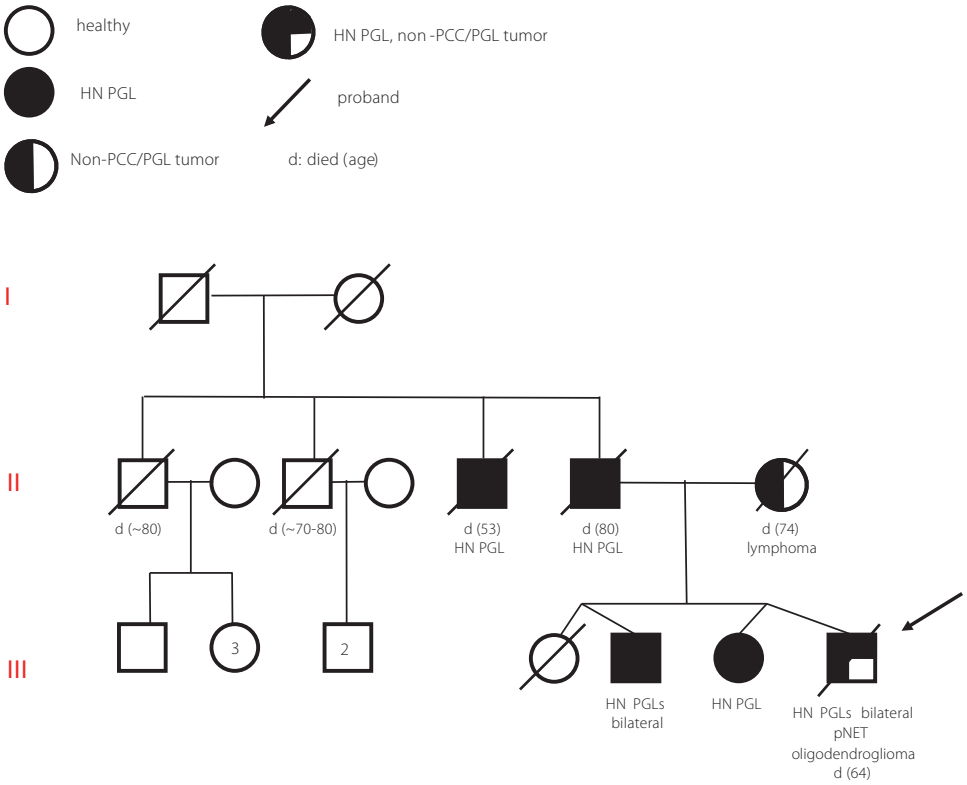

Figure 2. Pedigree from case 1, a patient with a germline *SDHD* (p.Asp92Tyr) mutation with bilateral paragangliomas, a pancreatic neuroendocrine tumor and an oligodendroglioma.

Abbreviations: *HN PGL* head and neck paraganglioma; *non-PCC/PGL* non-pheochromocytoma/paraganglioma; *pNET* pancreatic neuroendocrine tumor.

Discussion

Our initial immunohistochemical analysis of 35 nonparaganglionic tumors from 26 *SDH* mutation carriers identified and excluded 25 SDHB and SDHA immunopositive tumors. Immunohistochemical and molecular genetic data from eight SDHB immunonegative tumors confirm that a wide range of nonparaganglionic tumors fall within the SDH-related tumor spectrum and suggest the pancreatic NET may also expand this spectrum. In particular, we present strong supporting evidence indicating that the pancreatic NET described here very likely arose due to a germline *SDHD* mutation and is therefore a constituent of the *SDHD*-associated tumor spectrum. This is the first report of an association between a germline *SDHD* mutation and pancreatic NET and as such it expands the spectrum of hereditary pancreatic NETs, previously only attributable to multiple endocrine

neoplasia type 1 (MEN1), Von Hippel-Lindau disease (VHL), neurofibromatosis 1 (NF-1) and the tuberous sclerosis complex (TSC).[22]
The occurrence of a pancreatic NET in a *SDHD* mutation carrier is rare. Of the 239 patients with a germline *SDHD* mutation enrolled in the Leiden SDH Mutation Carrier Registry, only one of 22 patients suffering from a nonparaganglionic tumor was affected by a pancreatic NET. Despite the rarity, this finding might have potential implications for the surveillance of patients with a germline *SDHD* mutation. In The Netherlands, the surveillance protocol for *SDHD* mutation carriers includes urinary analysis for catecholamine excess every two years and MRI of the head/neck region every three years. Abdominal imaging is only advised when there is evidence of catecholamine excess. The addition of pancreatic NET to the SDH-related tumor spectrum suggests that it might be advisable to amend surveillance protocols, with the addition of standard abdominal imaging studies. Because the latter are not currently included in surveillance protocols in The Netherlands, the possibility that other patients in our registry carry undetected pancreatic NETs cannot be ruled out. However, given the rare occurrence rate in our study, further studies are needed to definitely amend surveillance protocols.

This study also included a patient with a germline *SDHA* mutation and an associated pituitary adenoma. This case, along with an additional case previously described by Papathomas *et al.*[12], suggests an important role for SDH mutations in hypophyseal tumorigenesis. These cases, together with the large patient cohort described by Dénes *et al.*[20], not only support a causative role of SDH genes in pituitary adenoma formation, but also highlight genotype-phenotype correlations in this fast-moving endocrine field. To date, 25 pituitary adenoma cases have been described occurring in association with confirmed germline *SDH* mutations/variants (Table 2).[12,17-20,23-26] Most of these tumors are prolactinomas, nonfunctioning adenomas or growth-hormone secreting macro-adenomas, with variable ages at diagnosis ranging from 15 to 84 years. It is now clear that germline *SDH* mutations are also a component of the familial spectrum of pituitary adenomas comprising *F*amilial *I*solated *P*ituitary *A*denoma (FIPA) (germline inactivating aryl hydrocarbon receptor interacting protein (*AIP*) mutations), Carney complex (germline inactivating *PRKARIA* mutations), *M*ultiple *E*ndocrine *N*eoplasia, type 1 (germline inactivating *MEN1* mutations) and *M*ultiple *E*ndocrine *N*eoplasia, type 4 (germline inactivating *CDKN1B* (p27/KIP1) mutations).[27-29]
Biallelic *SDHA* inactivation has been documented in both paragangliomic tumors and GISTs arising in patients harboring a germline *SDHA* mutation.[30] To the best of our knowledge, this is the first pituitary adenoma with proven biallelic inactivation in a patient with a germline *SDHA* mutation. Dwight *et al.* described a family in which a germline *SDHA* mutation was associated with a PGL in the proband, as well as a pituitary nonfunctioning macroadenoma in the proband's son. SDHA immunohistochemistry confirmed loss of expression in both

tumors. However, biallelic SDH inactivation was not detected in the pituitary adenoma; only paradoxical loss of the mutated allele was detected.[19] Dénes *et al.* demonstrated LOH in the pituitary adenomas of 3 *SDHB* patients, but there was ROH in the pituitary adenomas of two *SDHA*-mutated patients.[20] Gill *et al.* detected two inactivating SDHA mutations in a 62-year-old man with a prolactin-producing tumor, but neither of these mutations was present in the germline.[31] In an effort to identify the underlying pathogenic mechanism by which *SDH* mutations lead to pituitary tumor development, Xekouki *et al.* studied the pituitary in *Sdhb*$^{+/-}$ mice and provided evidence that pituitary hyperplasia in SDH-deficient cells may be the initial abnormality in the cascade of events leading to true adenoma formation.[26] These data unravel critical aspects related to hypophyseal pathobiology and further add to the understanding of the tumorigenic process.

In contrast, other tumor types, eg, bladder cancer, melanoma, prostate cancer and papillary thyroid cancer, retained SDHB/SDHA protein expression, suggesting that these tumors are not part of the SDH-associated tumor spectrum. To extend, the biological nature of heterogeneous breast cancer (case 3) in this particular genetic context (ie, *SDHD* and *BRCA-1*) remains elusive. Along these lines, the SDHB immunonegative abdominal ganglioneuroma (case 5) displayed ROH in the absence of additional mutations, strongly suggesting an alternative mechanism of SDHB protein loss other than loss of genomic regions encompassing the *SDHB* locus and/or a second 'exonic' somatic event. An alternative mechanism could be *SDHC* promoter hypermethylation.[32,33] Nevertheless, a limitation of the current study concerns the lack of methylation analysis for the promoter of *SDHC* gene.

In conclusion, the current study expands the SDH-related tumor spectrum and identifies pancreatic NET as a new component of this spectrum. This study also strengthens the etiological association of *SDH* genes with pituitary neoplasia, renal tumorigenesis and gastric GISTs as revealed in the Leiden SDH Mutation Carrier Registry. These findings may have implications for the surveillance protocol for patients with a germline *SDHD* mutation. In this context, further studies are warranted to elucidate the role of the disruption of the Krebs cycle in familial and sporadic pancreatic neuroendocrine tumorigenesis.

Table 2. Germline *SDH* mutations/variants and pituitary adenomas reported in the literature

Case	Age^/sex	Functional classification of PA	Germline *SDH* mutation	Biallelic SDH inactivation in PA	PGL/PCC	Reference
1	30/M	Pituitary nonfunctioning macroadenoma	*SDHA* c.1873C>T p.His625Tyr	Paradoxical loss (LOH) of the mutated allele	None	19
2	27/M	Pituitary prolactinoma, size NA	*SDHA* c.91C>T p.Arg31* *VHL*** c.589G>A p.Asp197Asn *AIP, MEN1* and *CDKN1B* are not available		PCC	20
3	49/F	Pituitary macroprolactinoma	*SDHA* c.91C>T p.Arg31*	p.D38V; somatic mutation as a second hit of biallelic inactivation	Bilateral HN PGL	present study
4	53/M	Pituitary nonfunctioning macroadenoma	*SDHA* variant c.969C>T p.Gly323Gly†	ROH	Abdominal PGL, Wilms tumor, retroperitoneal liposarcomas & renal oncocytoma	20
5	84/M	Pituitary GH-secreting macroadenoma	*SDHAF2* variant c.-52T>C		HN PGL	20
6	33/M¶	Pituitary macroprolactinoma	*SDHB* c.298T>C p.Ser100Pro	LOH	HN PGL	20
7	36/F¶	Pituitary macroprolactinoma	*SDHB* c.298T>C p.Ser100Pro		BAH	20
8	53/F	Pituitary nonfunctioning macroadenoma	*SDHB* c.587G>A p.Cys196Tyr	LOH	HN PGL	20
9	31/F	Pituitary macroprolactinoma	*SDHB* del ex 6 to 8	LOH		20
10	60/F	Pituitary macroprolactinoma	*SDHB* c.423+1G>A		HN PGL	20

Table 2. Germline *SDH* mutations/variants and pituitary adenomas reported in the literature *(Continued)*

Case	Age^/sex	Functional classification of PA	Germline *SDH* mutation	Biallelic SDH inactivation in PA	PGL/PCC	Reference
11	15/NA	Pituitary adenoma NA	*SDHB* c.761insC p.254fsX255		None	23
12	71/M#	GH-secreting adenoma	*SDHB* exon7 c.689G>A p.Arg230His		Bilateral HNPGL	26
13	51/F#	Pituitary microadenoma	*SDHB* exon 6 c.642+1G>A		Metastatic PGL GIST	26
14	60/M	Pituitary macoprolactinoma	*SDHC* c.256-257insTTT p.Phe85dup		HN PGL	18
15	53/M	Pituitary macroprolactinoma	*SDHC* c.380A>G p.His127Arg		HN PGLs	20
16	36/F	Pituitary macroprolactinoma	*SDHD* c.242C>T p.Pro81Leu		HN PGLs	24
17	41/M	Pituitary GH-secreting macroadenoma	*SDHD* c.298_301delACTC p.T100fsX133	LOH	HN- & ea-PGLs Bilateral PCCs	17
18	56/F	Pituitary GH-secreting macroadenoma	*SDHD* c.274G>T p.Asp92Tyr	ROH	HN PGLs	12 and present study
19	60/M	Pituitary macroprolactinoma	*SDHD* c.274G>T p.Asp92Tyr	LOH	HN PGLs PCC	12 and present study
20	NA	GH-secreting macroadenoma PGL1 syndrome	*SDHD* c.341A>G p.Tyr114Cys			25
21	NA	Pituitary nonfunctioning microadenoma PGL-1 syndrome	*SDHD* c.341A>G p.Tyr114Cys			25
22	NA	pituitary nonfunctioning microadenoma PGL-1 syndrome	*SDHD* c.341A>G p.Tyr114Cys			25
23	NA	Pituitary nonfunctioning microadenoma PGL-1 syndrome	*SDHD* c.341A>G p.Tyr114Cys			25

Table 2. Germline *SDH* mutations/variants and pituitary adenomas reported in the literature *(Continued)*

Case	Age^/sex	Functional classification of PA	Germline *SDH* mutation	Biallelic SDH inactivation in PA	PGL/PCC	Reference
24	40/F#	Pituitary macroprolactinoma	*SDHD* exon3 c.242C>T p.Pro81Leu		Bilateral PCC	26
25	51/F	Pituitary nonfunctioning macroadenoma	*SDHD* Asp92Tyr	NA, radiologic follow-up	HN PGL	present study***

Abbreviations: *SDH* succinate dehydrogenase; *PA* pituitary adenoma; *PGL* paraganglioma; *PCC* pheochromocytoma; *M* male; *F* female; *VHL* Von Hippel-Lindau; *MEN 1* multiple endocrine neoplasia type 1; *AIP* aryl hydrocarbon receptor interacting protein; *CDKN1B* cyclin-dependant kinase inhibitor 1B; *LOH* loss of heterozygosity; *ROH* retention of heterozygosity; *dup* duplication; *HN PGL* head and neck paraganglioma; *BAH* bilateral adrenal hyperplasia; *GH* growth hormone; *NA* not available; *ea-PGL* extra-adrenal paraganglioma; *fs* frame-shift; *GIST* gastrointestinal stromal tumor.

^ Age at diagnosis of the pituitary adenoma.

Age of diagnosis of the new syndromic association.

¶ These patients were first-degree relatives.

** This variant has been described in polycythemia vera but not in classical Von Hippel-Lindau syndrome.

*** One of the excluded patients in the present study, because no biopsy or surgically resected material was available.

† In silico splicing analysis software packages predicted that this variant may create a new splice donor site. RNA was extracted from peripheral blood using PAXgene Blood RNA Kit (PreAnalytiX, Hombrechtikon, Switzerland) but RT-PCR analysis found no evidence of aberrant splicing of the SDHA gene. Sequence analysis of DNA extracted from a paraffin embedded pituitary adenoma sample from this patient showed the presence of this variant with no evidence of loss of the normal allele in the tumor DNA when compared to the peripheral blood DNA. Tissue extracted from the father's nonfunctioning pituitary adenoma (NFPA) did not harbor the variant, while it was present in the germline DNA of the mother, suggesting that it is not the cause of NFPA in father and son. Its role in the proband's other tumors is unknown.

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Supplemental tables and figures

Supplemental Table 1. Clinicopathological and molecular genetic characteristics of nonparaganglionic tumors arising in *SDH* mutation carriers

Case	Age[a]/sex	Germline *SDH* mutation	Tumors observed (age at detection)	SDHB IHC	SDHA IHC	Second inactivation hit ¶	Status at last follow-up (age)
1	56/M	*SDHD* p.Asp92Tyr c.274G>T	pNET (56) Oligodendroglioma (57) GCT (L+R) (56) GVT (R) (56)	Neg Pos	Pos Pos	LOH#	Died (64)
2	61/M[b]	*SDHD* p.Asp92Tyr c.274G>T	Macro-prolactinoma (61) PCC (R) (61) GJTT (R) (60) GCT (L) (60) GVT (L+R) (60)	Neg	Pos	LOH	AWED (69)
3	38/F[c]	*SDHD* p.Pro81Leu c.242C>T	Breast cancer (38) GCT (L) (38)	Hetero-genous^	Pos	ROH	Died due to breast cancer (41)
4	55/F	*SDHD* p.Asp92Tyr c.274G>T	Gastric GIST (55) GCT (L+R) (50) GVT (L) (50)	Neg	Pos	LOH#	AWED (64)
5	42/M	*SDHB* c.423+1G>A	Abdominal ganglioneuroma (42) GVT (R) (42)	Neg	Pos	ROH	AWED (50)
6	45/M[d]	*SDHB* c.423+1G>A	RCC L foci 1 (45) RCC L foci 2 (45) RCC R (45) Gastric GIST (45) No PGL	Neg Neg Neg Neg	Pos Pos Pos Pos	LOH LOH LOH LOH	AWED (47)
7	49/F	*SDHA* p.Arg31X c.91C>T	Macroprolactinoma (49) GCT (R) (26) GCT (L) (49) Meningiomas (49)	Neg Pos	Neg Pos	p.D38V	AWD (meningi-omas) (65)
8	56/F[b]	*SDHD* p.Asp92Tyr c.274G>T	Pituitary adenoma, GH producing (56) GCT (L+R) (56) GJT (R) (56) GVT (R) (56)	Pos	Pos	ROH	Died (71)
9	70/M	*SDHD* p.Asp113fs c.337_340delGACT	Bladder cancer (70) Basal cell carcinoma (71) GCT (L+R) (NA) GJT (R) (NA)	Pos Pos	Pos Pos		Died (73)
10	48/F[e]	*SDHD* p.Asp92Tyr c.274G>T	Endometrial cancer (48) GCT (L+R) (45) GJT	Pos	Pos		AWED (59)

Supplemental Table 1. Clinicopathological and molecular genetic characteristics of nonparaganglionic tumors arising in *SDH* mutation carriers *(Continued)*

Case	Age[a]/sex	Germline *SDH* mutation	Tumors observed (age at detection)	SDHB IHC	SDHA IHC	Second inactivation hit ¶	Status at last follow-up (age)
11	40/F	*SDHD* p.Asp92Tyr c.274G>T	Breast cancer (40) GJT (R) (40) GVT (L) (51) Hodgkin lymphoma (18)	Pos	Pos		AWED (55)
12	57/M	*SDHD* p.Leu139Pro c.416T>C	Melanoma (57)	Pos	Pos		AWED (71)
13	57/F	*SDHD* p.Asp92Tyr c.274G>T	Breastcancer GCT (L+R) (65)	Pos	Pos		AWED (71)
14	57/M	*SDHD* p.Asp92Tyr c.274G>T	Melanoma shoulder (57) PCC (R) (55) GCT (L+R) (NA)	Pos	Pos		AWED (61)
15	68/M	*SDHD* p.Asp92Tyr c.274G>T	Prostate cancer (68) GJTT (L) (57) Abdominal PGL (65)	Pos	Pos		Died due to prostate cancer (71)
16	37/M	*SDHD* p.Asp92Tyr c.274g>T	Testicular cancer (37) Prostate cancer (53) GCT (L+R) (47) GJT (L) (47) GVT (L+R) (48)	Pos Pos	Pos Pos		AWED (62)
17	37/F	*SDHD* p.Asp92Tyr c.274G>T	Papillary thyroid carcinoma (37) GCT (L+R) (37) GVT (R) (37)	Pos	Pos		AWED (63)
18	56/M	*SDHD* p.Asp92Tyr c.274G>T	Bladder cancer (56) GCT (L) (38) GJTT (L) (38) GVT (R) (38)	Pos	Pos		AWD (63)
19	35/F	*SDHD* p.Leu139Pro c.416T>C	Breast cancer (R) (35) Breast cancer (L) (47) GCT (L) (41) GVT (R) (42)	Pos Pos	Pos Pos		AWED (49)
20	64/F	*SDHD* p.Asp92Tyr c.274G>T	Meningioma (64) Mediastinal PGL (67)	Pos	Pos		Died due to malignant PGL (74)
21	65/M	*SDHD* p.Leu139Pro c.416T>C	Prostate cancer (65) Gastric GIST (41) GCT (L+R) (57) GVT (L+R) (57) PCC (L) (62)	Pos NA	Pos NA		AWED (70)
22	50/F	*SDHD* p.Asp92Tyr c.274G>T	Meningeoma (50) Breast cancer (45) No PGL	Pos Pos	Pos Pos		AWED (55)

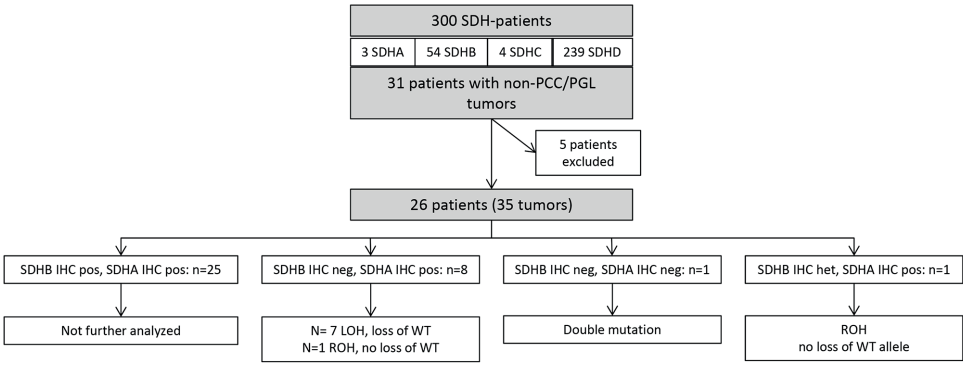
Supplemental Table 1. Clinicopathological and molecular genetic characteristics of nonparaganglionic tumors arising in *SDH* mutation carriers *(Continued)*

Case	Age[a]/sex	Germline *SDH* mutation	Tumors observed (age at detection)	SDHB IHC	SDHA IHC	Second inactivation hit ¶	Status at last follow-up (age)
23	46/F	*SDHB* c.423+1G>A	Basal cell carcinoma (46) No PGL	Pos	Pos		AWED (50)
24	33/F	*SDHB* c.423+1G>A	Breast cancer (33) PGL NA	Pos	Pos		Died due to breast cancer (49)
25	26/F	*SDHB* c.423+1G>A	Melanoma (26) No PGL	Pos	Pos		AWED (49)
26	48/M	*SDHB* p.Thr110Pro unclassified variant c.328A>C	Sebaceous gland carcinoma of the eyelid (48) No PGL	Pos	Pos		AWED (67)

Abbreviations: *M* male; *F* female; *IHC* immunohistochemistry; *pNET* pancreatic neuroendocrine tumor; *GCT* glomus caroticum tumor; *GVT* glomus vagale tumor; *GJT* glomus jugulare tumor; *GJTT* glomus jugulotympanicum tumor; *GTT* glomus tympanicum tumor; *PGL* paraganglioma; *PCC* pheochromocytoma; *RCC* renal cell carcinoma; *GIST* gastrointestinal stromal tumor; *L* left; *R* right; *GH* growth hormone; *AWD* alive with (non-paraganglionic) disease; *AWED* alive without evidence of disease other than head and neck PGL; *NA* not available; *ROH* retention of heterozygosity; *LOH* loss of heterozygosity; *LOH#* only one marker (centromeric or telomeric) was informative in each tumor as indicative of LOH. Sanger (direct) sequencing showed loss of the wild-type allele.

a Age at diagnosis of non-PCC/PGL tumor.
b Patient previously described by Papathomas *et al.*[12]
c Patient also carrier of a germline breast cancer 1 (*BRCA1)* mutation.
d Patient previously described by Gill A *et al.*[16]
e Also heterozygosity for mutation 467C>G, S156X (MSH-6).

¶ Loss of wild-type allele or somatic mutation.
^ Heterogenous is defined as granular cytoplasmic staining combined with a cytoplasmic blush lacking definite granularity or completely absent staining in the presence of an internal positive control throughout the same slide.



Supplemental Figure 1. Overview of the immunohistochemical and sequencing results from 35 nonparaganglionic tumors arising in 26 *SDH* mutation carriers.

Abbreviations: *SDH* succinate dehydrogenase; *PCC* pheochromocytoma; *PGL* paraganglioma; *IHC* immunohistochemistry; *pos* positive; *neg* negative; *het* heterogenous, *LOH* loss of heterozygosity; *ROH* retention of heterozygosity; *WT* wild-type.

Chapter 5

Sleep-mediated heart rate variability after bilateral carotid body tumor resection

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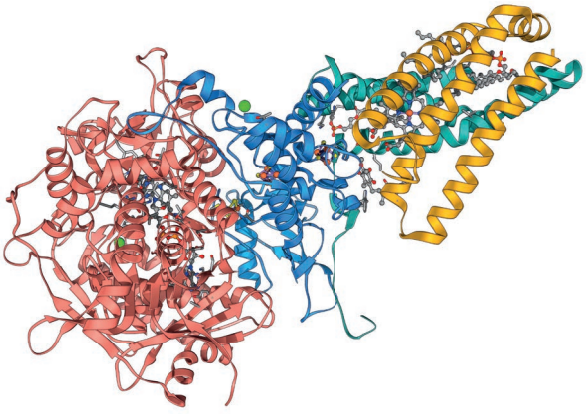

Sleep 2015 Apr 1;38:633-639

Abstract

Study Objectives: The carotid bodies are thought to play an important role in sleep-dependent autonomic changes. Patients who underwent resection of bilateral carotid body tumors have chronically attenuated baroreflex sensitivity. These subjects provide a unique opportunity to investigate the role of the baroreflex during sleep.

Design: One-night ambulatory polysomnography (PSG) recording.

Setting: At participants' homes.

Participants: Nine patients with bilateral carotid body tumor resection (bCBR) (four women, mean age 50.4 ±7.2 years) and nine controls matched for age, gender and body mass index.

Interventions: N/A

Measurements: Sleep parameters were obtained from PSG. Heart rate (HR) and its variability were calculated using 30-s epochs.

Results: In bCBR patients, HR was slightly but not significantly increased during wake and all sleep stages. The effect of sleep on HR was similar for patients and controls. Low frequency (LF) power of the heart rate variability spectrum was significantly lower in bCBR patients in active wakefulness, sleep stage 1 and rapid eye movement (REM) sleep. No differences were found between patients and controls for high frequency (HF) power and the LF/HF ratio.

Conclusions: bCBR is associated with decreased LF power during sleep, suggesting impaired baroreflex function. Despite this, sleep-related HR changes were similar between bCBR patients and controls. These findings suggest that the effects of sleep on HR are predominantly generated through central, non-baroreflex mediated pathways.

Keywords: Heart rate variability, carotid body tumor, paraganglioma, sleep, baroreflex

Introduction

Physiological sleep-dependent autonomic changes result from a complex interaction of peripheral cardiovascular reflexes and central modulation.[1,2] The baroreflex arc, with arterial baroreceptors mainly located in the carotid sinuses and aortic arch, is considered to be the critical relay in this complex integration.[3] Baroreflex sensitivity is continuously modulated and differs markedly between behavioral and physiological conditions, including sleep.[1,3,4] During non-rapid eye movement (non-REM) sleep, a progressive decrease is seen in peripheral sympathetic nerve activity and blood pressure (BP), together with a decrease in heart rate (HR).[1,2,5,6] The latter sign suggests increased parasympathic vagal activity. Conversely, a net increase of HR and BP has been reported during rapid eye movement (REM) sleep.[1,2,5,6] This increase is accompanied by irregular changes in autonomic activity.[1]
Paragangliomas are rare neuroendocrine tumors of paraganglia, which are neural-crest derived chromaffin tissues associated with the autonomic nervous system.[7] Paragangliomas in the head and neck region can arise from the carotid body, vagal body or jugulotympanic tissue (i.e. paraganglioma of the temporal bone).[8,9] Due to their location in close proximity to important neurovascular structures, tumor growth may lead to serious morbidity and cranial nerve impairment. These tumors can be removed without recurrence.[10] However, branches of the carotid sinus nerves may not be spared. Bilateral carotid body tumor resection (bCBR) may thus result in arterial baroreflex dysfunction.[11] Patients with bCBR are known to have significant lower baroreflex sensitivity compared with controls, i.e., a less marked heart rate response to a given rise or fall in blood pressure.[11] Baroreflex failure, whether from carotid endarteriectomy,[12] head and neck irradiation,[13,14] mixed cranial nerve neuroma,[15] neurosarcoidosis,[16] or brain stem stroke,[17] is associated with changes in heart rate variability (HRV). Notably, these patients have little 'low frequency' (LF) power, an index of baroreflex-mediated HR control.[18,19] This parallels findings in mouse models, where carotid sinus denervation resulted in lower values of LF power and baroreflex sensitivity.[20] So far, no data are available on the effects of sleep on HRV following bCBR. These patients provide a unique opportunity to study the role of the baroreflex in sleep. We therefore monitored HR and HRV during nocturnal sleep in bCBR patients and compared them with controls matched for age, gender, and body mass index (BMI).

Methods

Subjects

We included nine patients who had previously been treated with bCBR. These patients were recruited from the outpatient clinics of the departments of Endocrinology, Otorhinolaryngology and Surgery of the Leiden University Medical Center and through

an advertisement on the website of the Dutch paraganglioma patient network. For each patient we recruited a healthy control subject matched for gender, age (+/- 5 years), and BMI (+/- 3 kg/m^2).

Exclusion criteria were the presence of a pheochromocytoma, extra-adrenal paraganglioma, history of a psychiatric disorder, history of a diagnosed sleep disorder, or the use of sleep medication.

The Medical Ethics Committee of the Leiden University Medical Center approved the study protocol. All subjects provided written informed consent prior to the study.

Study Design

Polysomnography

Sleep was recorded at home using a portable polysomnography recorder (Somnologica Version 5.1.1, Embla, CO, USA). The measurement started at 16:00 and lasted for 24 hours. Duration of 'active wakefulness', sleep stages 1-3, and REM sleep were assessed. Active wakefulness was defined as all wake epochs occurring between the period from 16:00 until 20 minutes before onset of nocturnal sleep, and the period from 20 minutes after awakening in the morning until the end of the measurement.

Sleep stages and apnea/hypopnea events were manually scored in 30-s epochs by an experienced sleep technician, according to the guidelines of the American Academy of Sleep Medicine.[21] The polysomnography were analysed by a technician who was blinded to the diagnosis of the subject. The autonomic parameters were analyzed automatically.

Respiration was monitored with a nasal pressure sensor and two elastic bands (thorax and abdomen). Oxygen saturation was assessed continuously with a pulse oximeter attached to the index finger. Apneas were defined as a drop in the peak thermal sensor excursion by ≥90 % of baseline for longer than 10 s. Hypopneas were defined as a drop in the nasal pressure signal excursion by ≥30 % of baseline for longer than 10 s, with a ≥4 % desaturation from the pre-event baseline.

Electrocardiography and respiration

A continuous wavelet transform was implemented in Matlab (Version 13.1, Mathworks, MA, USA) to detect R-peaks in the electrocardiogram.[22] A filter was used to exclude outliers, with outliers defined as values that differed > 25 beats/min from the previous or next sample. The signal was resampled at 5 Hz.

R-peak detection resulted in a series of consecutive R-R intervals, split into consecutive 30-s epochs for analysis. For each epoch the mean HR was calculated and a frequency spectrum by creating time-frequency domains through fast Fourier transform. From the frequency spectrum, the LF (0.04-0.15 Hz) and high frequency (HF) (0.15-0.4 Hz) power component

were calculated. The HF component is considered to represent vagal activity, and the LF component to reflect baroreflex-mediated sympathetic activity.[18,23] LF/HF ratio was computed as a reflection of the sympathovagal balance.

Autonomic parameters

LF power and HR were selected as main outcome parameters. Secondary outcome measures included HF and LF/HF ratio.

Selection of epochs

All epochs of sleep following onset of nocturnal sleep and prior to awakening in the morning were included in our analysis. For active wakefulness, a selection of epochs was made, as the length of the active wakefulness period proved to exceed those of the sleep states considerably, which could affect the results. We therefore nullified this effect by limiting the number of epochs of active wakefulness to that of stage 2 epochs for each subject. The wake epochs were selected in a random fashion. To account for the effects of arousals, we labeled every epoch following a transition from sleep stage 3 to stage 2 or 1 and sleep stage 2 to stage 1. We defined these epochs as 'arousal transitions'. In addition, we identified all epochs that coincided with either an apnea or a hypopnea to study the autonomic effects of respiratory arousals.

Statistical Analysis

Overall we included an average total number of 1269 epochs per subject. For each epoch the autonomic parameters, sleep/wake stage, subject number, beta-blocker use, presence of bCBR, arousal transitions and apnea/hypopnea (Figure 1) were recorded and entered into our model. For each autonomic parameter a linear mixed effects regression model was formed to describe the effect of sleep stages and bCBR on the autonomic parameters. To obtain a normal distribution of the residuals of the models, a natural logarithm-transformation was applied. Sleep stage, beta-blocker use, apneas/hypopneas, arousal transitions and the interaction between bCBR and sleep stages were entered as fixed and subjects as random effects to the model. Because of expected differences in variability between sleep stages and between patients and controls, the random subject effects were stratified for sleep stage/wakefulness (5 levels) and patient status (2 levels).

Outliers with a standardized residual at a distance greater than 2.5 standard deviations from 0 were excluded from the linear mixed effects regression model.

P-values below 0.05 were considered to be significant. All statistical analyses were performed using R (Version 3.0.0, R).

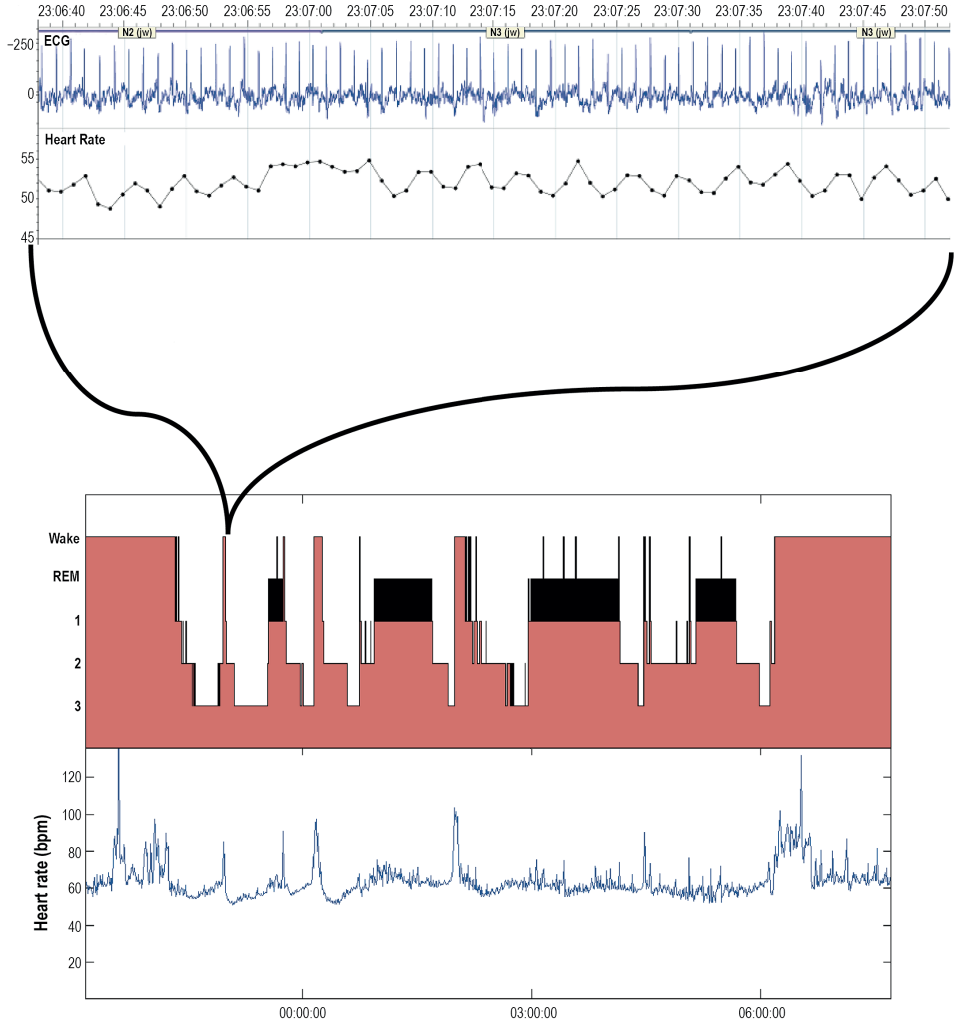

Figure 1. Schematic diagram illustrating our data analysis. For each subject, we analysed the heart rate data during overnight sleep and a random selection of active wakefulness (lower part of the figure). Overall we included an average total number of 1269 epochs per subject. The upper part of the figure zooms in on a representative one-minute segment illustrating how the heart rate was calculated from the one-channel ECG data. For each epoch the autonomic parameters, sleep/wake stage, subject number, beta-blocker use, presence of bCBR, arousal transitions and apnea/hypopnea were recorded and entered into our model.

Results

Participant characteristics

The bCBR group comprised 6 patients with a mutation in subunit D of the *SDH* gene (*SDHD*), one obligate *SDHD* mutation carrier, one patient tested negative for germline mutations in *SDH* genes and one patient had not been genetically tested (Table 1). In the bCBR patients, resection of the first carotid body tumor (CBT) was performed 12.5±7.6 years (range 2.2–25.2 years) and resection of the second CBT 8.9±6.8 years (range 1.2–21.5 years) prior to current study. Three patients had additional head and neck paragangliomas. Two patients had a vagal body tumor and one patient had a jugular foramen tumor. Two patients used beta-blockers (bisoprolol 2.5 mg once daily and propranolol, unknown dosage). None of the participants were shift-workers or were known to have arrhythmias.

Table 1. Clinical characteristics of nine patients with bilateral carotid body tumor resection (bCBR) and their matched healthy controls

Characteristics	bCBR n=9	Controls n=9
Age (year)	50.4 ± 7.2	51.0 ± 8.2
Gender (n)		
Male	5	5
Female	4	4
BMI (kg/m2)	25.7 ± 2.8	25.0 ± 4.2
First CBR		
Time since (yrs; range)	12.5 ± 7.6 (2.2- 25.2)	-
Second CBR		
Time since (yrs; range)	8.9 ± 6.8 (1.2-21.5)	-

bCBR bilateral carotid body tumor resection; *BMI* body mass index; *CBR* carotid body tumor resection.
Data are shown as mean values ± standard deviation.

Sleep parameters

bCBR patients spent significantly more time in sleep stage 1 than controls (12.6% versus 7.5%; $p<0.05$). Apart from this difference, polysomnography parameters were similar between groups. As reported previously, no significant differences in apnea/hypopnea index between patients and controls were found (Table 2).[24]

Autonomic parameters

In bCBR patients, HR was slightly but not significantly increased during wake and all sleep stages (Table 3, Figure 2). The effect of sleep on HR was similar for patients and controls. LF power of the HRV spectrum was significantly lower in bCBR patients during active wakefulness ($p<0.05$), sleep stage 1 ($p<0.01$) and REM sleep ($p<0.05$). (Table 3, Figure 2). No differences were found between bCBR patients and controls for HF power and the LF/HF ratio.

Table 2. Polysomnography results of nine patients with bilateral carotid body tumor resection (bCBR) and their matched healthy controls

Polysomnography	bCBR n=9	Controls n=9
Total sleep time (min)	416.4 ± 52.2	441.8 ± 30.0
Sleep latency (min)	8.0 ± 4.3	4.8 ± 1.8
Sleep efficiency (%)	91.1 ± 3.7	91.8 ± 3.2
% Sleep stage 1	12.6 ± 6.0*	7.5 ± 2.0*
% Sleep stage 2	38.3 ± 8.6	38.4 ± 6.5
% Sleep stage 3	24.8 ± 6.3	27.4 ± 6.5
% REM sleep	24.3 ± 7.6	26.7 ± 3.7
Awakenings (/h)	1.8 ± 0.7	1.9 ± 0.8
AHI (/h)	4.0 ± 4.3	3.3 ± 3.8
AI (/h)	1.2 ± 1.1	1.7 ± 3.0
Hypopnea index (/h)	2.9 ± 3.8	1.6 ± 1.4
Oxygen desaturation events (/h)	4.2 ± 3.9	3.1 ± 3.2

Data are shown as mean values ± standard deviation.
bCBR bilateral carotid body tumor resection; *REM* rapid eye movement; *AHI* apnea hypopnea index; *AI* apnea index.
* p<0.05.

Table 3. Regression coefficients derived from the linear mixed effects model with the effects of sleep on cardiovascular parameters

Model Parameters	HR (bpm)	ln(LF)	ln(HF)	ln(LF/HF)
	β / S.E.	β / S.E.	β / S.E.	β / S.E.
intercept (active wakefulness)	74/3	1/0.2	-0.3/0.4	1/0.3
stage 1	-12/2***	-0.4/0.2*	-0.3/0.2	-0.1/0.2
stage 2	-16/2***	-1/0.2***	-0.6/0.2**	-0.4/0.2*
stage 3	-13/2***	-1/0.3***	-0.6/0.2*	-0.8/0.2**
stage REM	-13/2***	-0.9/0.2***	-0.9/0.2**	-0.1/0.2
beta-blocker	-7/9	-0.4/0.6	-1/1	0.4/0.9
apnea/hypopnea	0.2/0.2	0.5/0.0***	0.2/0.0***	0.3/0.0***
arousal transitions#	1/0.3**	0.5/0.0***	0.1/0.0***	0.3/0.0***
stage active:bCBR	6/6	-0.9/0.3*	-0.2/0.7	-0.6/0.5
stage 1:bCBR	7/5	-1/0.3**	-0.3/0.6	-0.7/0.5
stage 2:bCBR	8/5	-0.6/0.4	-0.0/0.6	-0.5/0.5
stage 3:bCBR	7/5	-0.6/0.4	-0.1/0.7	-0.5/0.5
stage REM:bCBR	7/5	-1/0.4*	-0.4/0.7	-0.6/0.5

Abbreviations: *HR* heart rate; *bpm* beats per minute; *ln* natural log-transformed; *LF* low frequency; *HF* high frequency; β regression coefficient; *S.E.* standard error of the regression; *REM* rapid eye movement; *bCBR* bilateral carotid body tumor resection.

* p <0.05; ** p<0.01; *** p<0.001; # defined as transitions from sleep stage 2 to 1 and from sleep stage 3 to 2 or 1.

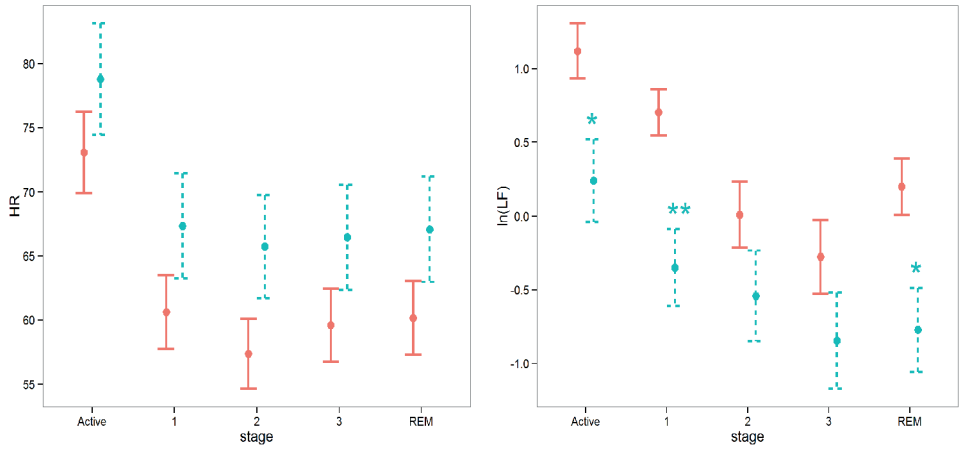

Figure 2. Effects of sleep on heart rate (HR) and low frequency (LF) power of heart rate variability in nine patients with bilateral carotid body tumor resection (dashed, blue lines) and their matched healthy controls (solid, red lines). * p <0.05; ** p<0.01. Bars represent standard error of the mean.

Abbreviations: *HR* heart rate; *REM* rapid eye movement; *ln* natural logarithm; *LF* low frequency power; *Active* active wakefulness.

Discussion

We found that the LF component of HRV was significantly lower during active wakefulness, sleep stage 1, and REM sleep in bCBR patients compared to controls, reflecting baroreflex dysfunction. Interestingly, in spite of these signs of baroreflex dysfunction, the effect of sleep on HR was similar in bCBR patients and their matched controls. These findings suggest that the sleep-related HR decrease primarily results from non-baroreflex mediated pathways.

Sleep studies

Patients with bCBR spent significantly more time than controls in sleep stage 1. This increase in light sleep could not be explained by an increased prevalence of sleep-disordered breathing.[24] Whether these findings are of clinical importance is disputable, as no differences were found in measures of daytime sleepiness between bCBR patients and controls.[24]

Baroreflex and sleep

As in previous daytime studies, bCBR patients had a lower LF indicating baroreflex failure.[12-17] Differences in LF power during sleep between bCBR patients and controls were most apparent in sleep stage 1 and during REM sleep. During deeper sleep (sleep stages 2 and 3), this difference was less marked. This contrast could not be attributed to an *increase* in LF during deeper sleep in the bCBR patients: only minimal LF changes were seen throughout

sleep stages (Figure 2). Instead, the healthy controls appeared to have a marked *decrease* in LF activity during sleep stages 2 and 3 compared to sleep stage 1 and REM sleep. Accordingly, previous work indicated that sleep stages 2 and 3 are associated with the lowest values of sympathetic outflow in healthy controls[1,5,6,25] The absence of significant differences in LF power between patients and controls during sleep stages 2 and 3 is thus explained by the transient suppression of baroreflex function during normal deep sleep. Baroreflex function is thus state-dependent, meaning that it is differently modulated by central influences in the different sleep phases and by wake adaptive behaviours.[1,3,26]

In spite of the marked contrasts in baroreflex function during sleep stage 1 and REM sleep in bCBR patients, sleep-related HR changes were similar for bCBR patients and controls. Notably, the relative higher LF values of the controls seen during sleep stage 1 and REM compared to the bCBR patients, did not translate to more marked HR contrasts in these sleep stages (Figure 2). Also, within the healthy controls we found that sleep stage 3 was associated with lowest LF values, while HR was similar between both sleep stage 2 and 3. This confirms previous work on sleep-related sympathetic outflow: sleep stage 3 was associated with a consistently lower value in muscle sympathetic nerve activity whereas HR remained stable between both sleep stages 2 and 3.[1,5,6,25] Taken together, these findings suggest that the sleep-related HR changes primarily result from non-baroreflex mediated pathways. Which alternative pathways should be considered? It could be argued that the HR decrease during sleep results from inactivity. However, the gradual decrease of HR seen in different non-REM sleep stages and the contrasting effects in REM sleep favor central modulation. Accordingly, overnight infusion of vasopressive drugs (phenylephrine) in healthy subjects results in a sustained decrease in blood pressure the following morning, thus suggesting that overnight blood pressure increases are counteracted by central mechanisms.[27,28] Thus, while inactivity may, of course, in part contribute, this cannot explain the complex dynamics between sleep stages. Diurnal contrasts in autonomic control could also result from neuroendocrine changes, e.g., circadian rhythms in adrenocorticotropic activity and the renin-angiotensin-aldosterone system (RAAS). Clear circadian patterns have been identified for HR and its variability.[29] The overall effects of these circadian effects, however, appear to be modest and can only partly explain the sleep-related HR changes. Supporting this view, the blood pressure dipping pattern has shown to be primarily related to sleep-wake phases rather than endogeneous circadian oscillators.[26] The close correlation between HR and sleep stages argues for direct effects of the sleep-wake cycle on the central autonomic network. Sympathetic outflow decreases and baroreflex sensitivity increases along with the depth of non-REM sleep.[1,5,6,25] Sleep-related autonomic alterations may thus well fit in the general concept of sleep as a state of adaptive inactivity.[30] The central autonomic network during non-REM sleep may involve the hypothalamic ventrolateral preoptic area, central thermoregulatory and central baroreflex pathways, and command neurons in the pons

and midbrain.[26,31] The intact sleep-related HR decrease in bCBR patients suggest that the peripheral baroreceptors play a minor role in the cardiovagal modulation during sleep. We speculate that the balance of neuronal autonomic control changes throughout the sleep-wake cycle. While awake, baroreceptors have an important role in buffering circulatory oscillations induced by activity and mental stress. During non-REM sleep, these oscillations decrease. Consequently, the influence of the baroreceptors gradually declines and autonomic outflow is predominantly driven by the central autonomic network[26,32] (Figure 3). During REM sleep, both pathways are likely equally important: centrally induced transient augmentations of sympathetic outflow cause an increase in baroreceptor activity.[1,2,5,6]

Limitations

We did not quantify baroreflex function as we lacked continuous blood pressure measurements and did not perform daytime standardized baroreflex tests. The low LF values in our bCBR patients are however a clear indication of baroreflex dysfunction.[18,19] Accordingly, a previous small study in eight bCBR subjects confirmed that bCBR causes chronic impairment of baroreflex control of both heart rate and sympathetic nerve activity.[11] Also, ideally given the complex nature of the autonomic nervous system measurements should be multimodal (e.g., including muscle sympathetic nervous activity, sympathetic skin response, pulse arterial tonometry (PAT)) to account for regional differences.

The small sample size of our study was inevitable in view of the extremely low prevalence of paragangliomas. The study did not have enough power to detect small differences. Therefore we were not willing to correct for multiple testing; this may have caused a type I error. However, we believe that our conclusions are valid, as the direction of the results were consistent and in line with previous daytime studies.[12-17] Again, because of the small number of subjects we included two patients who were using beta-blockers. To overcome this limitation, we corrected for beta-blocker use in our mixed effects model, but no significant effects were observed. Another limitation is the lack of measurements of leg movements. We are not aware of an increased prevalence of periodic leg movements in bCBR patients. Even if such a difference would have been the case, the effects on our outcome parameters would be likely minimal as leg movements only have short-term effects: autonomic parameters did not differ between patients with periodic leg movements and controls if the periodic leg movements epochs were excluded.[33]

Conclusion

In conclusion, the arterial baroreceptors are a critical relay in the autonomic network modulating both the peripheral and the central autonomic outflow. Our small study in patients with probable baroreflex failure, however, indicates that the sleep-related HR decrease predominantly results from non-baroreflex mediated, central mechanisms.

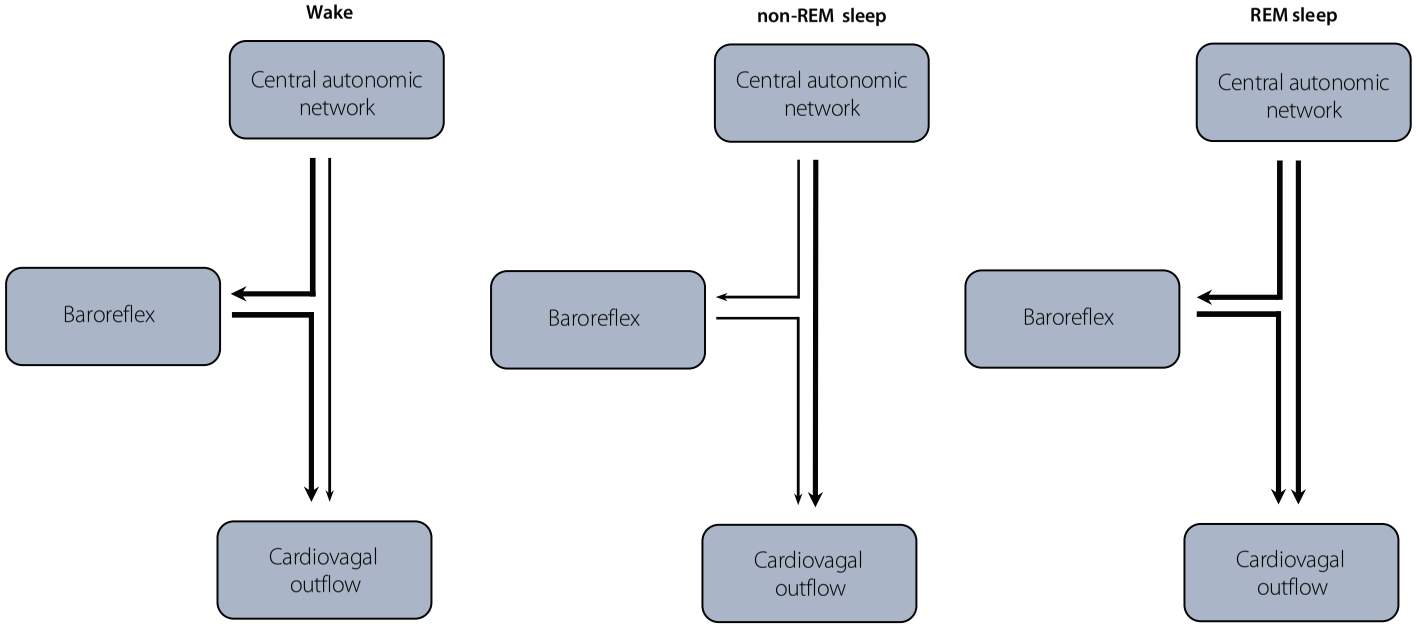

Figure 3. Simplified schematic diagram illustrating the major changes in neuronal autonomic control throughout the sleep/wake cycle. While awake, cardiovagal outflow is predominantly controlled by peripheral baroreceptors, whereas during non-REM sleep the central autonomic network becomes the driving force. During REM sleep, both pathways are likely equally important: central induced transient increases of sympathetic outflow cause an increase in baroreceptor activity.

Abbreviations

bCBR	bilateral carotid body tumor resection
BMI	body mass index
BP	blood pressure
BPM	beats per minute
EEG	electroencephalographic
HF	high frequency
HR	heart rate
HRV	heart rate variability
LF	low frequency
non-REM	non rapid eye movement
PLMS	periodic leg movements
REM	rapid eye movement
SDHD	succinate dehydrogenase subunit D

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Chapter 6

^{131}I-MIBG therapy for malignant paraganglioma and pheochromocytoma: systematic review and meta-analysis

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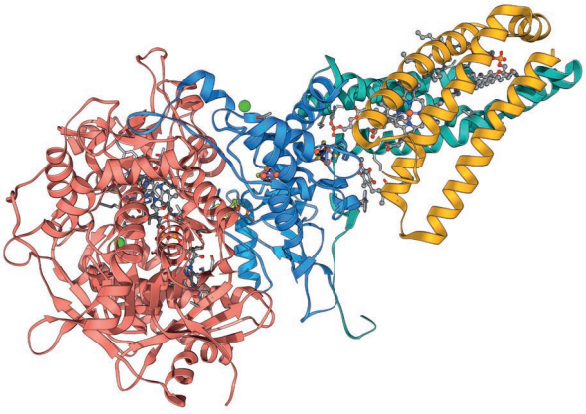

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Abstract

Background: ^{131}I-MIBG therapy can be used for palliative treatment of malignant paraganglioma and phaeochromocytoma. The main objective of this study was to perform a systematic review and meta-analysis assessing the effect of ^{131}I-MIBG therapy on tumour volume in patients with malignant paraganglioma/phaeochromocytoma.

Methods: A literature search was performed in December 2012 to identify potentially relevant studies. Main outcomes were the pooled proportions of complete response, partial response and stable disease after radionuclide therapy. A meta-analysis was performed with an exact likelihood approach using a logistic regression with a random effect at the study level. Pooled proportions with 95% confidence intervals (CI) were reported.

Results: Seventeen studies concerning a total of 243 patients with malignant paraganglioma/phaeochromocytoma were treated with ^{131}I-MIBG therapy. The mean follow-up ranged from 24 to 62 months. A meta-analysis of the effect of ^{131}I-MIBG therapy on tumour volume showed pooled proportions of complete response, partial response and stable disease of, respectively, 0·03 (95% CI: 0·06–0·15), 0·27 (95% CI: 0·19–0·37) and 0·52 (95% CI: 0·41–0·62) and for hormonal response 0·11 (95% CI: 0·05–0·22), 0·40 (95% CI: 0·28–0·53) and 0·21 (95% CI: 0·10–0·40), respectively. Separate analyses resulted in better results in hormonal response for patients with paraganglioma than for patients with phaeochromocytoma.

Conclusions: Data on the effects of ^{131}I-MIBG therapy on malignant paraganglioma/phaeochromocytoma suggest that stable disease concerning tumour volume and a partial hormonal response can be achieved in over 50% and 40% of patients, respectively, treated with ^{131}I-MIBG therapy. It cannot be ruled out that stable disease reflects not only the effect of MIBG therapy, but also (partly) the natural course of the disease.

Introduction

Background

Paragangliomas (PGLs) are rare vascular, neuroendocrine tumours (NETs) of paraganglia. They derived from either sympathetic chromaffin tissue in adrenal and extra-adrenal locations or from parasympathetic tissue of the head and neck (HNPGL).[1] Approximately, 80% of PGLs arise from the adrenal medulla and are referred to as phaeochromocytoma (PCC).[1,2] Although the majority of PGLs are benign, there is a risk of malignant degeneration of 10% for PCC and 10–20% for extra-adrenal non-HNPGLS.[3] Malignant disease is defined as the presence of metastatic lesions at sites where neuroendocrine tissue is normally absent.[4-6] The prognosis in malignant PGL/PCC is known to be poor and treatment remains basically palliative. The overall 5-year survival in patients with malignant PGL/PCC is <50%.[3,7,8] In 1984, Sisson *et al*.[9] reported their first experience with radionuclide therapy using ^{131}I-MIBG in the treatment of patients with malignant PCC. Since then, several studies have investigated the therapeutic option of radiolabelled MIBG in the treatment of malignant PGL/PCC, however, with varying success rates.

At present, the precise effect of ^{131}I-MIBG therapy for the treatment of malignant PGL/PCC is unclear. One systematic review on the effect of radionuclide therapy in malignant NETs has been published,[10] however, results were not stratified for PGL/PCC and a meta-analysis assessing this effect has never been performed.

Objective of the study

The aim of this study was to perform a systematic review and meta-analysis of the effects of ^{131}I-MIBG therapy on tumour volume in malignant PGL/PCC. Secondary objectives were to assess biochemical response, overall survival, progression-free survival and toxicity.

Materials and methods

Eligibility criteria

Studies assessing the effect of ^{131}I-MIBG therapy on tumour volume of malignant PGL/PCC were eligible for inclusion. Malignant PGL/PCC was defined as the presence of metastases in non-neuroendocrine tissue distant to the primary tumour.[4-6] Studies concerning patients with nonmalignant PGL/PCC according to this definition were excluded, for example, locally invasive PGL/PCC without metastases, unless data for patients with metastatic PGL/PCC could be extracted separately.

The analysis aimed to assess the proportion of PGL/PCC patients with tumour response after ^{131}I-MIBG therapy, with biochemical response (i.e. levels of catecholamines and/or their metabolites), overall survival, progression-free survival and toxicity as secondary outcomes.

According to the 'Response evaluation in solid tumours (RECIST) criteria' version 1.1, a partial treatment response is defined as 'at least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters'.[11] However, the RECIST criteria have not (yet) been widely accepted in the field of PGLs. Therefore, it was decided not to restrict inclusion of studies to RECIST criteria only for tumour response. The same applies to biochemical response: we included both papers that measured biochemical response with clear criteria as well as papers that measured biochemical response without clear definitions.

To accurately assess response rates, only studies determining treatment response (tumour volume) by radiologic imaging were eligible for inclusion. Furthermore, only studies reporting a population of five or more patients with PGL/PCC were included, to avoid the inclusion of cases or case series that are more prone to selection and publication bias. In case of multiple studies describing the same cohort, the study that comprised the highest number of subjects and/or the longest duration of follow-up was included.

To strengthen the results and enlarge the number of patients with these relatively rare tumours that could be included in our systematic review and meta-analysis, we also included studies that described patients with other types of NETs if data for patients with metastatic PGL/PCC could be extracted separately and if these studies reported a population of five or more patients with PGL/PCC.

Eligible studies were restricted to languages familiar to the authors (English, French, German and Dutch). When reported data were not sufficient for accurate data extraction, we tried to contact the authors for clarification.

Search strategy

In December 2012, PubMed, MEDLINE, EMBASE, Web of Science, COCHRANE, CINAHL, Academic Search Premier and ScienceDirect were searched to identify potentially relevant studies (search strategy provided upon request). References of key articles were assessed for additional relevant articles.

Data extraction

All studies obtained from the search strategy were entered into reference manager software (Reference Manager version 12; Thomson Reuters, Philadelphia, PA, USA) and were screened on title and abstract. Potentially relevant studies were retrieved for detailed assessment. For eligible studies, data were independently extracted by two reviewers (LvH and NN). Disagreements between reviewers were resolved by consensus, but when doubt remained, a third reviewer (EC) decided.

Risk of bias assessment

The present meta-analysis is based on observational studies. Risk of bias assessment was based on study components that potentially bias an association between the intervention under study (^{131}I-MIBG therapy) and the primary outcome (tumour volume). The following elements were assessed for all studies:

1. Risk of selection bias. Inclusion of consecutive exposed patients or a random sample of the inception cohort was considered a low risk of bias.
2. Adequacy of reporting of intervention (^{131}I-MIBG therapy). When number of doses and activity of ^{131}I-MIBG therapy were reported, this was considered adequate.
3. Adequacy of measurement of tumour volume. The effect of ^{131}I-MIBG therapy on tumour volume should have been measured by either sequential MRI or CT scanning.
4. Adequate definition of tumour response. A prespecified definition of objective tumour response was considered adequate.
5. Adequacy of follow-up. Loss to follow-up <5% was considered to represent a low risk of bias.

Statistical analysis

The main outcome of the present meta-analysis was the pooled proportion of PGL/PCC patients with tumour response after ^{131}I-MIBG therapy. The pooled proportion of PGL/PCC patients with biochemical response after ^{131}I-MIBG therapy was the secondary outcome. For all studies, the proportion of PGL/PCC patients with tumour response was calculated as the number of PGL/PCC patients with tumour response divided by the total number of PGL/PCC patients treated with ^{131}I-MIBG therapy. The same procedure was applied to the proportion of PGL/PCC patients with biochemical response. For all proportions, exactly, 95% confidence intervals (95% CI) were calculated.

Meta-analysis was performed using an exact likelihood approach. The method used was a logistic regression with a random effect at the study level.[12] Given the expected clinical heterogeneity, a random effects model was performed by default, and no fixed effects analyses were performed. For meta-analysis of proportions, the exact likelihood approach based on a binomial distribution has advantages compared with a standard random effects model that is based on normal distributions.[13] Firstly, estimates from a binomial model are less biased than estimates from models based on a normal approximation.[14] This is especially the case for proportions that are close to 0 or 1. Secondly, no assumptions are needed for the exact approximation when dealing with zero cells, whereas the standard approach needs to add an arbitrary value (often 0·5) when dealing with zero cells. Adding values to zero cells is known to contribute to the biased estimate of the model.[15] All analyses were performed with stata 12.0 (Stata Corp, College Station, TX, USA).

Results

Study selection

The initial search resulted in 1648 unique records; 93 were selected for detailed assessment. After detailed assessment, 76 articles were excluded for the following reasons: outcome other than tumour response (n = 25), no radiologic evaluation or unclear if radiologic evaluation was performed (n = 7), no original data (n = 5), article included patients with various NETs without stratification for patients with PGL/PCC (n = 3). Twenty-three studies were excluded because the number of patients with PGL/PCC did not exceed five and three studies did not meet our criteria for malignant PGL/PCC. Furthermore, ten studies comprised a cohort also described in another publication; the studies with the smallest sample sizes were excluded.[7,16-24] No new articles were found in references of key articles. Finally, a total of 17 studies were included in the present analysis, 16 written in English and one written in French.[9,25-40]

Study characteristics

Study characteristics are displayed in Table 1. Included studies were published from 1984 to 2012. All included studies were classified as cohort studies.[41] A total of 243 patients were included in this meta-analysis. The largest study contained 49 subjects. One study included patients only after there was evidence of progressive disease,[26] while in most other studies, patients were included irrespective of evidence of progressive disease or secretory symptoms, or it was not reported.

Mean age ranged from 25 to 49 years. Mean duration of follow-up ranged from 24 to 62 months.

Patients were treated with a median total administered activity of ^{131}I-MIBG ranging from 6882 to 39400 megabecquerel, MBq (186–1065millicurie, mCi), with a median number of infusions ranging from 1 to 7.

Table 1. Characteristics of included MIBG studies

First author (year)	Number of patients	Mean age ± SEM (years)	Primary tumor localization (*n*)	Prior treatment (*n*)	Median total administered activity in MBq (range)	Median number of infusions (range)	Imaging modality used to determine response	Tumor markers	Mean ± SEM duration of follow-up (months)	Loss of follow-up, *n*+ reason
Sisson (1984)[9]	5	40.2 ± 10.0	PCC (5)	Surgery (5)	13801 (9990-17908)	3 (2-4)	CT	Plasma catecholamines (n.s.), urinary catecholamines (n.s.), VMA, MN, NM	n.r.	n.r.
Charbonnel (1988)[27]	12	Range 28-65	PCC (12)	n.r.	Range 1850-62197	Range 1-8	CT	Urinary and/or plasma catecholamines (n.s.) and/or urinary VMA and/or urinary metanephrines	Range 6-24	n.r.
Krempf (1991)[32]	15	48.8 ± 3.4	PGL (2) PCC (13)	Surgical resection of primary tumor (15)	18500 (11100-85900)	4 (2-11)	CT ^{131}I-MIBG	Urinary catecholamines (n.s.), metanephrines (n.s.), VMA	26.3 ± 3.5	1, reason n.r.
Lewington (1991)[33]	13	n.r.	PCC (13)	n.r.	Mean 4300 (5 patients) Mean 23400 (8 patients)	n.r.	Combined CT/MRI/MIBG	Urinary catecholamines (n.s.)	n.r.	n.r.
Schlumberger (1992)[38]	10[a]	40.2 ± 5.9 at time of diagnosis	PCC (10)	Resection of primary tumor (9) - plus resection of metastases (5) - plus chemotherapy (1)[b]	6882 (3552-23088)	2 (1-5)	CT	Urinary DA, E, NE, VMA, MN, NM, MT, HVA	n.r.	1, reason n.r.

Table 1. Characteristics of included MIBG studies *(Continued)*

First author (year)	Number of patients	Mean age ± SEM (years)	Primary tumor localization (*n*)	Prior treatment (*n*)	Median total administered activity in MBq (range)	Median number of infusions (range)	Imaging modality used to determine response	Tumor markers	Mean ± SEM duration of follow-up (months)	Loss of follow-up, *n*+ reason
Sakahara (1994)[37]	5	44.2 ± 9.3	PGL (2) PCC (3)	Resection of primary tumor (5)	7400 (3700-10730)	2 (1-3)	Scintigrams with whole-body or spot images/CT	Serum catecholamines (n.s.)	24.0 ± 6.8	none
Hartley (2001)[31]	6	40.8 ± 5.7	PGL (1) PCC (5)	Surgery (2) Surgery and chemotherapy (2) Surgery and radiotherapy (1) Surgery, radiotherapy and chemotherapy (1)	25900 (7400-44400)	3.5 (1-6)	CT/X-ray	Urinary catecholamines (n.s.)	n.r.	1, referred for 90Y-labelled octreotide therapy
Mukherjee (2001)[34]	15	44.1 ± 4.1 (18-68)	PGL (7) PCC (8)	Chemotherapy (2)[c]	22200 (7400-47400)	3 (1-7)	n.s.	Urinary DA, E, NE, VMA, MN	61.8 ± 13.4	1, moving abroad
Bomanji (2003)[25]	6	38. 8 ± 3.3	PGL (6)	Surgery and chemotherapy (3) Surgery, radiotherapy and ^{131}I-MIBG (1) Radiotherapy (1)	33670 (12654-36519)	3 (1-3)	CT ^{123}I-MIBG	Urinary VMA and catecholamines (n.s.)	n.r.	0
Safford (2003)[36]	33	Median 50 (range 27-77)	PGL (11) PCC (22)	n.r.	Mean 20313, range 2590-45251	1 (1-6)	MIBG-scan/CT/MRI	Urinary DA, E, NE, VMA, MN	Median 33.6, range 2-114	n.r.
Gedik (2008)[29]	19	45.9 ± 3.0	PGL (7) PCC(12)	Radiotherapy (4) Chemotherapy (3) Chemo-+radiotherapy (1) Surgery (18)	22200	3 (1-10)	CT/MRI/ ^{131}I-MIBG	Urinary VMA, metanephrines, normetanephrines, free catecholamines (E, NE, DA) Serum catecholamines (n.s.)	36.5 ± 6.5	n.r.

Table 1. Characteristics of included MIBG studies *(Continued)*

First author (year)	Number of patients	Mean age ± SEM (years)	Primary tumor localization (*n*)	Prior treatment (*n*)	Median total administered activity in MBq (range)	Median number of infusions (range)	Imaging modality used to determine response	Tumor markers	Mean ± SEM duration of follow-up (months)	Loss of follow-up, *n*+ reason
Gonias (2009)[30]	49[d]	Median 42.6 (range 10.3-64.4) at entry of study	PGL (34) PCC(15)	Chemotherapy (15) Radiation (16) Surgery (44)	1st treatment 30266 (18204-42920) range all treatments 18204-118067	1 (1-3)	CT/MRI/ ^{123}I-MIBG	Serum CgA, urinary fractionated catecholamines and metanephrines (n.s.)	Median 24, range 1.2-180	1, reason n.r.
Castellani (2010)[26]	24[e]	Total[f] 36.3±3.3 *Group 1* 24.8±4.5 *Group 2* 44.8±3.2 age at diagnosis	Total: PGL (11) PCC(13) *Group 1* PGL (7) PCC(3) *Group 2* PGL (4) PCC(10)	*Group 1* Surgery (5)Surgery and chemotherapy (2), Surgery, chemotherapy and radiotherapy (2) *Group 2* Surgery (10) Surgery, chemotherapy and radiotherapy (1) Surgery and Sandostatine (1) Chemotherapy (2)	*Group 1* 39400 (5500-66600) *Group 2* 24100 (9200-57200)	*Group 1* 7 (1-12) *Group 2* 2 (1-6)	CT/MRI/^{131}I-MIBG	Catecholamine values n.s.	*Group 1* median 49.8, range 2.4-228 *Group 2* median 27.6 range 2.4-90	n.r.
Shilkrut (2010)[39]	10	48.0 ± 5.2	PGL (3) PCC (7)	Surgery plus external beam radiation (1)	11200 (3700-22400)	2 (1-4)	CT	Serum catecholamines and urinary catecholamine metabolites (n.s.)	24.1 ± 5.1	n.r.
Rachh (2011)[35]	10[g]	33.8 ± 6.0	PGL (4) PCC (6)	Surgery, chemotherapy/ radiotherapy (10)	11785 (7363-15614)	2 (1-4)	MIBG-scan/CT	Urinary VMA	29.5 ± 9.1	5, reason n.r.

Table 1. Characteristics of included MIBG studies *(Continued)*

First author (year)	Number of patients	Mean age ± SEM (years)	Primary tumor localization (*n*)	Prior treatment (*n*)	Median total administered activity in MBq (range)	Median number of infusions (range)	Imaging modality used to determine response	Tumor markers	Mean ± SEM duration of follow-up (months)	Loss of follow-up, *n*+ reason
Szalat (2011)[40]	6	35.8 ± 5.2 at time of diagnosis	PGL (1) PCC (5)	Resection of primary tumor and metastases (5) -plus Thalidomide-IFN-indomethacine and Imatinib (1)	n.r.	2 (1-4)	CT/MRI/ ^{18}F-FDG-PET/ ^{18}F-DOPA-PET	Urinary E, NE, NM Blood CgA	n.r.	n.r.
Fishbein (2012)[28]	5[h]	34.6 ± 4.0 age at initial diagnosis	PGL 5	Chemotherapy (1)	74/kg per cycle	1 (1-3)	'Imaging': n.s.	Plasma MN, NMN, DA, E, NE, CgA	n.s.	0

Abbreviations: *n.r.* not reported; *n.s.* not specified; *MBq* megabecquerel; *PGL* paraganglioma; *PCC* pheochromocytoma; *CT* computed tomography; *MRI* magnetic resonance imaging; *MIBG* metaiodobenzylguanidine; *FDG-PET* fluorodeoxyglucose positron emission tomography; *DOPA-PET* dihydroxyphenylalanine positron emission tomography; *DA* dopamine; *E* epinephrine; *NE* norepinephrine; *VMA* vanillylmandelic acid; *MN* metanephrines; *NM* normetanephrines; *MT* methoxytyramine; *HVA* homovanilic acid; *CgA* chromogranin A.

a Originally, 13 patients were included. Three patients were already included in the study of Krempf *et al.*[32]; we excluded data from these patients.

b Four patients received radiotherapy. It was unclear if this was before or after MIBG-therapy.

c Thirteen patients received additional therapy with surgery, radiotherapy, chemotherapy, octreotide or hepatic artery embolization. One patient received chemotherapy in between two cycles of ^{131}I-MIBG therapy.

d Fifty patients enrolled and treated, 1 lost to follow up, data given only of 49 patients.

e Originally 28 patients were included, however after contact with the authors it was clarified that four patients (2 PGL and 2 PCC) did not have metastatic disease; we excluded (if possible) data from these patients. Cumulative activity, number of infusions of ^{131}-I-MIBG therapy and duration of follow-up were only given of all 28 patients.

f Low (group 1) versus intermediate (group 2) activity regimes were compared.

g Originally, 12 patients were included. Two patients did not have metastatic disease; we excluded data from these patients.

h Patients were treated with sequential ^{131}I-MIBG and external beam radiotherapy because of areas of symptomatic, bulky tumor.

Risk of bias assessment

Summary characteristics of the risk of bias assessment are shown in Table 2. In 15 studies (88%), included patients were explicitly described as consecutive exposed patients or as a random sample of the inception cohort. The intervention under study (i.e. ^{131}I-MIBG therapy) was adequately described in 13 studies (76%).

Table 2. Risk of bias assessment

First author (Year)	Consecutive patients or random sample of inception cohort	Determination of intervention adequately reported	Adequate measurement of tumor response	Adequate definition of tumor response	Number of patients lost to follow-up (%)
Sisson (1984)[9]	Unclear	Yes	Yes	No[a]	n.r.
Charbonnel (1988)[27]	Unclear	No[b]	Yes	Yes	n.r.
Krempf (1991)[32]	Yes	Yes	Yes	Yes	1 (7%)
Lewington (1991)[33]	Yes	Yes	Yes	No[a]	n.r.
Schlumberger (1992)[38]	Yes	Yes	Yes	Yes	1 (8%)
Sakahara (1994)[37]	Yes	Yes	Yes	No[a]	0
Hartley (2001)[31]	Yes	Yes	No[c]	Yes	1 (17%)
Mukherjee (2001)[34]	Yes	Yes	No[d]	Yes	1 (7%)
Bomanji (2003)[25]	Yes	Yes	Yes	Yes	0
Safford (2003)[36]	Yes	Yes	Yes	Yes	n.r.
Gedik (2008)[29]	Yes	Yes	Yes	Yes	n.r.
Gonias (2009)[30]	Yes	No[e]	Yes	Yes	1 (2%)
Castellani (2010)[26]	Yes	Yes	Yes	Yes	n.r.
Shilkrut (2010)[39]	Yes	Yes	Yes	Yes	n.r.
Rachh (2011)[35]	Yes	Yes	Yes	Yes	5 (50%)
Szalat (2011)[40]	Yes	No[f]	Yes	Yes	n.r.
Fishbein (2012)[28]	Yes	No[g]	No[d]	Yes	0

Abbreviations: *n.r.* not reported; *n.s.* not specified.
a Prespecified definitions for assessment of tumor response not reported.
b Total administered activity: only range reported.
c Radiological responses assessed by CT/X-rays; unclear which patients were evaluated by CT and which by X-ray.
d Specific imaging modality not reported.
e Administered activity of MIBG only reported for the first treatment.
f Administered activity not reported.
g Total dose not reported.

The effect of therapy on tumour volume was adequately measured (i.e. by CT and/or MRI) in 14 studies (82%). In two studies, the specific imaging modality was not reported and one study also used X-rays to assess tumour response and did not specify which imaging

modality was performed in which patients. Three studies did not report prespecified definitions for assessment of tumour response. Actual loss to follow-up was reported in 9 of 17 studies (53%). In five of these 9 studies, loss to follow-up exceeded 5%.

Effect of ^{131}I-MIBG therapy on tumour volume

Table 3 gives an overview of reported outcomes after ^{131}I-MIBG therapy. To assess tumour response, eight studies used the World Health Organization (WHO) criteria,[25,27,29,31,32,34,36,38] four studies the RECIST criteria, of which one version 1.1,[28,30,39,40] one study the Eastern Cooperative Oncology Group (ECOG) criteria[26] and one study the International Neuroblastoma Response Criteria (INRC).[35] Three studies did not report how tumour response was assessed.[9,33,37] Proportions of complete response after ^{131}I-MIBG therapy ranged from 0·00 to 0·38. For partial response, this was 0·00 to 0·83 and for stable disease 0·00 to 0·90.

Results of the random effects meta-analysis are displayed in Fig. 1. Pooled proportions of complete response, partial response and stable disease were 0·03 (95% CI: 0·06–0·15), 0·27 (95% CI: 0·19–0·37) and 0·52 (95% CI: 0·41–0·62), respectively.

Effect of ^{131}I-MIBG therapy on catecholamine excess

Hormonal response was measured by all studies except one.[40] Five studies assessed hormonal response using the World Health Organization (WHO) criteria,[25,27,29,34,36] one study using the ECOG criteria[26] and one study using the INRC.[35] Five studies did not use common criteria[30-32,38,39] and four studies did not report prespecified definitions for assessment of hormonal response.[9,28,33,37] Proportions of complete response ranged from 0·00 to 0·27, partial response from 0·16 to 1·00 and stable disease from 0·00 to 0·63.

The random effects meta-analysis resulted in pooled proportions of complete response, partial response and stable disease of respectively 0·11 (95% CI 0·05–0·22), 0·40 (95% CI 0·28–0·53) and 0·21 (95% CI 0·10–0·40) (Fig. 2).

Paraganglioma vs phaeochromocytoma

To assess potential differences in response between PGL and PCC, separate analyses were performed for these two subgroups. Unfortunately, in three manuscripts, the results were not separately reported for PCC and PGL,[30,32,36] resulting in a total of 99 patients with PCC and 47 patients with PGL for separate analyses. Results are displayed in Figs 1 and 2. For tumour response, pooled proportions of complete response and partial response were slightly larger for patients with PGL than for patients with PCC (respectively, 0·04 and 0·30 for patients with PGL *vs* 0·01 and 0·28 for patients with PCC), with a larger proportion of stable disease in patients with PCC (0·50 for patients with PCC *vs* 0·28 for patients with PGL). Concerning catecholamine excess, results of the meta-analysis showed better response rates for patients with PGL than for patients with PCC.

Table 3. Outcomes of MIBG

First author (year)	Response on imaging			Response hormonal			Overall survival time (confidence interval)	Progression-free survival time (confidence interval)	Toxicity (*n*)
	Complete response (*n*)	Partial response (*n*)	Stable disease (*n*)	Complete response (*n*)	Partial response (*n*)	Stable disease (*n*)			
Sisson (1984)[9]	0[a]	2 (40%)	3 (60%)	0[a]	3 (60%)	0	n.r.	n.r.	Transient leucopenia (1) Thrombocytopenia (1) Herpes zoster (1) Alopecia (1) Subendocardial infarct (1)
Charbonnel (1988)[27]	0[b]	3 (25%)	n.r.	2 (17%)[b]	5 (42%)	0	n.r.	n.r.	Bone marrow hypoplasia (1) Transient increase in bloodpressure and catecholamines (3) Transient increase in transaminases and AF, reversible (2)
Krempf (1991)[32]	0[b]	5 (33%)	7(47%)	4[c] (27%)	3 (20%)	6 (40%)	Probability of survival: 48 at 22 months, 40 at 28 months	n.r.	Slight and transient rise in BP and catecholamines (3), diarrhea (2), ALT/AST twice normal for 1 week (1) grade 2 trombocytopenia (2), grade 3 leukopenia (2) pancytopenia grade 4 (1)[d]
Lewington (1991)[33]	0[e]	4 (31%)	5 (38%)	0[f]	8 (62%)	0	n.r.	n.r.	Thrombocytopenia (1) Marrow aplasia (1)
Schlumberger (1992)[38]	0[b, g]	1 (17%)	1 (17%)	0[g]	1 (17%)	1 (17%)	n.r.	n.r.	Grade 2 thrombocytopenia (1)[h] Grade 1 thrombocytopenia (1)
Sakahara (1994)[37]	0[i]	2 (40%)	2 (40%)	0[i]	3 (60%)	2 (40%)	n.r.	n.r.	Orthostatic hypotension, hypertension and hyperglycaemia (1)

Table 3. Outcomes of MIBG *(Continued)*

First author (year)	Response on imaging			Response hormonal			Overall survival time (confidence interval)	Progression-free survival time (confidence interval)	Toxicity (*n*)
	Complete response (*n*)	Partial response (*n*)	Stable disease (*n*)	Complete response (*n*)	Partial response (*n*)	Stable disease (*n*)			
Hartley (2001)[31]	0[b]	0	4 (67%)	0[j, k]	1 (20%)	3 (60%)	n.r.	n.r.	Grade 3 thrombocytopenia (1)[h] Hypertensive encephalopathy after diagnostic scanning (1)
Mukherjee (2001)[34]	0[l]	8 (53%)	4 (27%)	2 (22%)[l]	6 (67%)	1 (11%)	n.r.	n.r.	Bone marrow suppression and premature ovarian failure (1) Mild hepatic dysfunction (1) Other complications n.s.
Bomanji (2003)[25]	0[m]	5 (83%)	0	1 (25%)[n]	2 (50%)	1 (25%)	n.r.	n.r.	n.s.
Safford (2003)[36]	8 (38%)[b, o]	8 (38%)	n.r.	0[p]	12 (60%)	0	Median 4.7 years 5 year survival rate 45%[q]	n.r.	Bone marrow suppression (4) Nausea/Vomiting (2) Herpes zoster (1)
Gedik (2008)[29]	0[r]	8 (47%)	6 (35%)	2 (17%)[r]	6 (50%)	4 (33%)	Median survival time 42 months	Mean 28.5 ± 5.7 months	Grade 3 thrombocytopenia (2)[s] Grade 2 neutropenia (1) Grade 3 thrombocytopnia and grade 1 neutropenia (1) Grade 4 neutropenia and Grade 4 thrombocytopenia (1) Diarrhea (1) Nausea (1) Palpitations and dizziness (1)

Table 3. Outcomes of MIBG *(Continued)*

First author (year)	Response on imaging			Response hormonal			Overall survival time (confidence interval)	Progression-free survival time (confidence interval)	Toxicity (*n*)
	Complete response (*n*)	Partial response (*n*)	Stable disease (*n*)	Complete response (*n*)	Partial response (*n*)	Stable disease (*n*)			
Gonias (2009)[30]	4 (9%)[t, u]	8 (18%)	24 (53%)	6 (19%)[v,w]	5 (16%)	5 (16%)	5-year overall survival 64% ± 9%	n.r.	Grade 3-4 neutropenia in 87%, grade 3-4 thrombocytopenia 83%, grade 3-4 anemia 8%.[s] Grade 3-4 non hematologic toxicity: ARDS (2) BOOP (2) MDS (2) Grade 3 acute hypertension (7) Hypogonadism (4) Pulmonary embolism (1) Infection (1) Pneumonia (1) Hyperthyroidism (3)
Castellani (2010)[26]	*Group 1*[x] 1 (10%) *Group 2*[y] 2 (15%)	*Group 1* 2 (20%) *Group 2* 3 (23%)	*Group 1* 6 (60%) *Group 2* 5 (38%)	*Group 1*[z] 0 *Group 2*[z] 1 (17%)	*Group 1* 3 (43%) *Group 2* 3 (50%)	*Group 1* 3 (43%) *Group 2* 0	n.r.	n.r.	*Group 1*[aa] Grade 2 thrombocytopenia (2) Hypothyroidism (2) *Group 2* 'Grade 2 toxicity'(2) Grade 3 thrombocytopenia (3) Grade 3 neutropenia (2) Grade 4 thrombocytopenia (3) Grade 4 neutropenia (2) Grade 4 anemia (1) Myelofibrosis (1)
Shilkrut (2010)[39]	0[bb]	3 (33%)	5 (56%)	0[bb]	5 (56%)	0	n.r.	Mean 23.1 ± 5.0 months	Thrombocytopenia grade 1 (1)[s] Subclinical hypothyroidism (2) Vomiting (2)

Table 3. Outcomes of MIBG *(Continued)*

First author (year)	Response on imaging			Response hormonal			Overall survival time (confidence interval)	Progression-free survival time (confidence interval)	Toxicity (*n*)
	Complete response (*n*)	Partial response (*n*)	Stable disease (*n*)	Complete response (*n*)	Partial response (*n*)	Stable disease (*n*)			
Rachh (2011)[35]	0[cc]	1 (10%)	9 (90%)	1 (13%)[cc]	2 (25%)	5 (63%)	n.r.	n.r.	n.s.
Szalat (2011)[40]	0[dd]	0	5 (83%)	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Fishbein (2012)[28]	0[ee]	0	0	0[ff]	3 (100%)	0	n.r.	n.r.	n.r.

Abbreviations: *n.r.* not reported; *n.s.* not specified; *AF* alkaline phosphatase; *ALT* alanine aminotransferase; *AST* aspartate aminotransferase; *ARDS* acute respiratory distress syndrome; *BOOP* bronchiolitis obliterans organizing pneumonia; *MDS* myelodysplastic syndrome.

a Reduction in tumor volume and function of tumors to less than 50% of pretreatment values.
b Tumor response according to the World Health Organization (WHO) criteria.[57]
c Hormonal response was defined as: Complete response (CR): normalization of all urine tests; Partial response (PR): 50% or greater reduction; No change (NC); Progressive disease (PD): 25% or greater increase in all urine hormone values.
d Toxicity according to the WHO criteria.[57]
e Two patients <50% tumor reduction, 2 patients >50% tumor reduction.
f "Biochemical improvement"; defined as catecholamine excretion to at least 50% of baseline levels in those who had elevated catecholamine excretion.
g Response evaluated in 6 patients. Hormonal response defined as: CR: normalization of all urinary tests; PR: 50% or greater reduction in urinary measurements, with duration measured from the initiation of ^{131}I-MIBG therapy; NC; PD: 25% or greater increase in urinary measurements.
h Criteria for grading toxicity not reported.
i No specified definition of response reported.
j Hormonal response defined as: CR: urinary catecholamines in the normal range; PR: values at the end of treatment <50% of the starting value.
k One patient was non-secreting.
l Two patients with PR received radiotherapy almost simultaneously: not possible to differentiate response between ^{131}I-MIBG therapy and radiotherapy. Tumor response and hormonal response assessed by WHO criteria[56]with some modifications. Hormonal response assessed in 9 patients with secretory tumors (6 PCC and 3 PGL).

m Tumor response based on the WHO tumor response criteria[57] with some modifications.
n Two patients were non-secreting. Hormonal response defined according to the WHO tumor response criteria.[57]
o Radiographic evaluation of response obtained in 22 patients. Eight patients showed 'response'; not specified.
p Hormone levels measured in 20 patients. Response defined by the WHO criteria.[57]
q Some patients received additional radiation or chemotherapy regimens. There was no difference in survival in those who received additional radiation therapy; there was an increased survival in patients who received additional chemotherapy.
r Radiographic evaluation available in 17 patients (7 PGL and 10 PCC), hormonal response in 12 (2 PGL and 10 PCC). Tumor response and hormonal response according to the WHO criteria[57].
s Toxicity grading according to the NCI CTCAE version 3.0.
t Tumor response defined by the RECIST criteria.[58]
u After first treatment. Assessed in 45 patients.
v Tumor response defined as: PR: decreases ≥50%; PD: increases >20%. Overall, tumor marker response was a PR if all markers achieved at least a PR, was a PD if any marker indicated PD, was an SD if all modalities maintained SD and was a minor response if any marker achieved PR or CR while other markers remained SD.
w After first treatment. Assessed in 32 patients.
x Radiological responses were graduated on the basis of Eastern Cooperative Oncology Group (ECOG) criteria.[59] Two with a partial response >50%. Assessed in 10 patients.
y Assessed in 13 patients (4 PGL and 9 PCC). Two with a partial response <50%, one with a partial response >50%. Response defined by ECOG criteria.[59]
z In Group 1 and group 2, respectively, seven (4 PGL and 3 PCC) and six (2 PGL and 4 PCC) patients with secreting tumors were evaluable.
aa Toxicity only reported for all 28 included patients. Toxicity graded according to the ECOG criteria.[59]
bb Response evaluated in 9 patients(2 PGL and 7 PCC). Tumor response defined by the RECIST criteria.[58] Hormonal response defined as response (normalization or >50% decrease in all measured catecholamines and its metabolites) or no response. It was not reported which of the 5 patients had normalization of catecholamines and which a decrease; all these patients are categorized under 'partial response', since at least a partial response was achieved.
cc Hormonal response obtained in 8 patients (2 PGL and 6 PCC). Tumor response defined by the International Neuroblastoma Response Criteria (INRC).[60]
dd Response defined by the RECIST criteria.[58]
ee Response defined by the RECIST criteria version 1.1.[11]
ff Response assessed in 3 patients, described as 'decreased plasma metanephrines and catecholamines' not specified. All these patients are categorized under 'partial response', since at least a partial response was achieved.

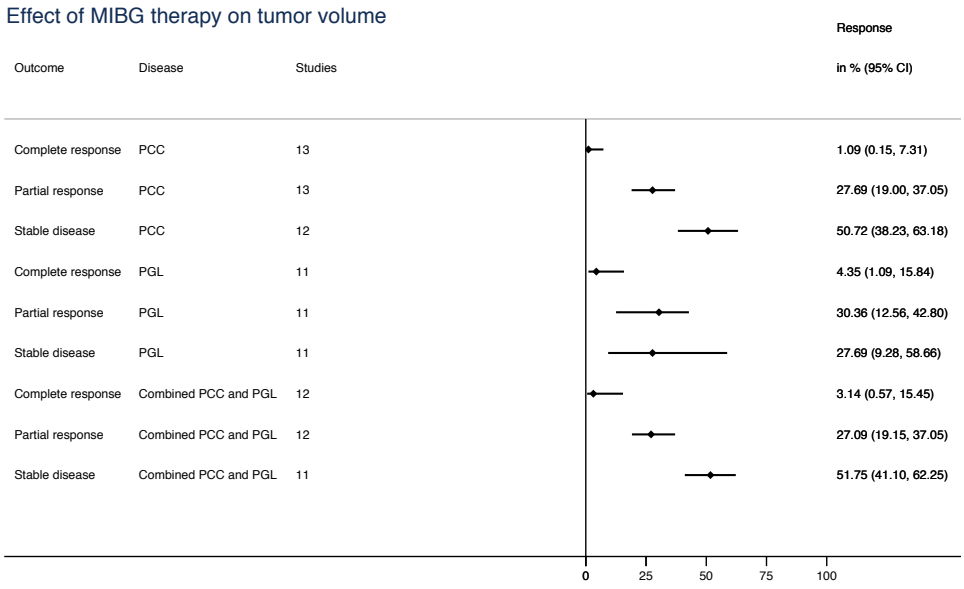

Figure 1. Meta-analysis, tumor volume.

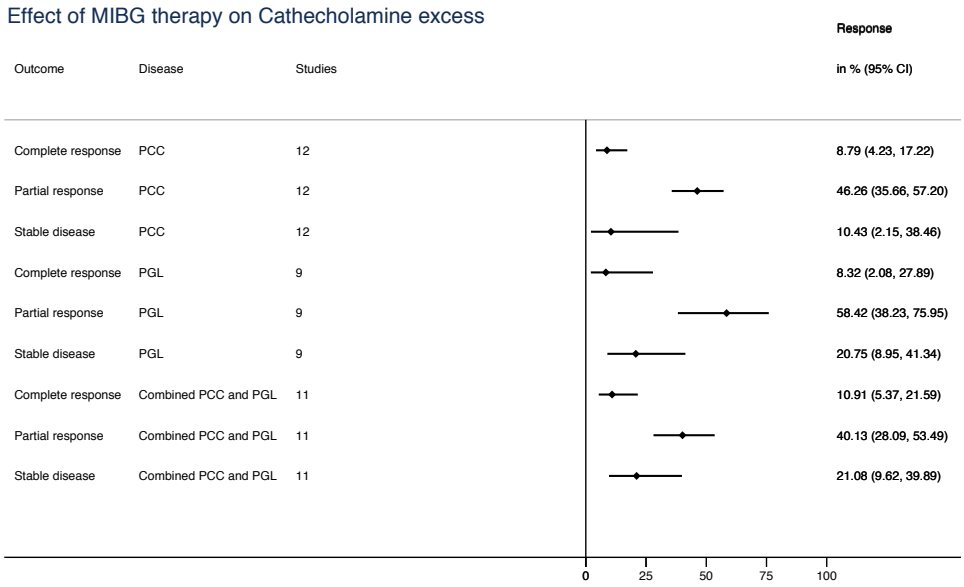

Figure 2. Meta-analysis, catecholamine excess.

Survival and side effects of ^{131}I-MIBG therapy

Two studies reported a 5-year survival rate of 45% and 64%[30,36] and two studies a mean progression-free survival time of 23·1 and 28·5 months, respectively.[29,39]

To grade toxicity, three studies used the ctcae version 3.0 [29,30,39] one study describes the ECOG criteria[26] and another study WHO criteria.[32] Eight studies did not specify toxicity criteria. In two studies, which also included patients with other types of NETs, toxicity could not be extracted for the patients with PGL/PCC. Another two studies did not report toxicity. Haematologic toxicity was the most frequently reported side effect, with grade 3–4 neutropenia occurring in up to 87% of treatments and grade 3–4 thrombocytopenia in 83%. Renal toxicity was not reported.

Discussion

The present systematic review and meta-analysis aimed to assess tumour response of malignant PGL/PCC after ^{131}I-MIBG therapy. Although reported therapy effects varied considerably, our meta-analysis showed that stable disease following ^{131}I-MIBG therapy could be achieved in 52% of the patients and a partial hormonal response in 40%. Reported 5-year survival rates were 45% and 64% and mean progression-free survival times 23·1 and 28·5 months. The most frequent side effect was haematologic toxicity.

By including only patients with metastatic PGL/PCC in our meta-analysis, we tried to abolish incorrectly positive tumour response rates. With the results of this study, it is possible to inform both patients with malignant PGL/PCC and their treating physicians more adequately concerning the expected tumour response after ^{131}I-MIBG therapy.

In patients with malignant PGL/PCC, in whom treatment is basically palliative, stable disease following therapy may be a useful criterion for assessing tumour response, especially in patients with previously demonstrated progressive disease. However, in the included studies, the protocol when to initiate treatment differed widely. One study included patients only after there was evidence of progressive disease,[26] while most other studies included patients irrespective of evidence of progressive disease. Therefore, the possibility remains that stable disease is not merely a therapy effect, but also a reflection of the natural course of the disease with slow progression in a subset of patients.

The same applies to hormonal response: included studies did not report if patients were selected for inclusion irrespective of catecholamine excess. However, as reduced tumour function and, as a consequence, symptom palliation are important treatment goals in patients with malignant PGL/PCC, it is a meaningful finding that our meta-analysis showed that a partial hormonal response after ^{131}I-MIBG therapy can be achieved in 43% of patients. Future studies may translate these results by including quality of life measures as an end-point when assessing results of ^{131}I-MIBG therapy.

There are some other limitations that should be taken into account when interpreting this meta-analysis. The included studies displayed differences in treatment regimens; patients treated with ^{131}I-MIBG therapy received a total administered activity ranging from 6882 to 39400 MBq (186–1065 mCi), with a mean number of infusions ranging from 1 to 7. This may have contributed to the variation in reported response rates. One study addressed this issue; Castellani *et al*.[26] compared the use of low *vs* intermediate activity per session of ^{131}I-MIBG therapy and found that increasing the single session activity resulted in a shorter median time to achieve a significant response, which was obtained with a lower median cumulative activity in a lower median number of sessions. It is unclear if a higher total administered activity results in a higher number of patients with tumour response. However, as the amount of administered activity does not reflect the (radiation) dose that is actually absorbed in the tumour, pretherapeutic individual tumour dosimetry should be performed to optimize dose delivery. To achieve this, more clinical studies to obtain reliable dosimetry of radiation therapy for malignant PGL/PCC are needed.

Included studies also displayed heterogeneity in assessing the effects of radionuclide therapy. Several different criteria were applied to define tumour response. In addition, the effect of therapy on tumour volume was not adequately measured (i.e. by CT and/or MRI) in three studies. This may have contributed to the variety in reported response rates. Future research on the effects of radionuclide therapy on malignant PGL/PCC should apply uniform criteria for changes in tumour size and sequential CT and/or MRI scanning to objectively assess treatment response.

In our systematic review, we included a total of 243 patients, of which 94 were PGL patients. In two of these patients with PGL, a HNPGL was the primary tumour. Although HNPGLs are classified as parasympathetic PGLs, which differ from sympathetic PGL in terms of secretion and of receptor expression profile, both HNPGL patients showed pretherapeutic uptake of the radiopharmaceutical on diagnostic ^{123}I-/^{131}I- MIBG scintigraphy. Therefore, these patients were good candidates for MIBG therapy, and it seems unlikely that the inclusion of these two patients has skewed our results.

Paraganglioma/phaeochromocytoma 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).[42,43] Although the different susceptibility genes vary in their risk of developing malignant PGL/PCC,[6,44] to our knowledge, it is not known if the response to ^{131}I-MIBG therapy differs between tumours of different genetic backgrounds. Only four of the included studies reported known gene mutations, resulting in a total of 15 *SDHB* patients, three patients with neurofibromatosis type I, one patient with MEN2A and one with MEN2B.[26,28,30,38] Gonias *et al*.[30] found that patients with an *SDHB* mutation were more likely to achieve complete or partial remission. *SDHB* mutation was not, however, predictive of overall survival or event free survival. Unfortunately, it is

not reported if all other included patients tested negative for susceptibility genes or were not genetically tested at all. Furthermore, the small sample sizes in these studies prohibit a separate meta-analysis.

Possibly, differences in genetic background may also explain the higher hormonal response rates in patients with PGL than in patients with PCC. Examining different responses to ^{131}I-MIBG therapy between PGL/PCC patients of different genetic backgrounds would be an interesting topic for further research.

In the last few years, an increasing number of metastatic NETs have been treated with peptide receptor radionuclide therapy (PRRT) with radiolabelled somatostatin analogues like ^{177}Lutetium (Lu)-DOTA-octreotide and ^{90}Yttrium (Y)-DOTA-lanreotide.[45] Differentiated neuroendocrine cancers frequently express subtypes of the somatostatin receptor family[45,46]; PGL/PCC were found to express predominantly subtypes 2A and 3, and therefore, patients with PGL/PCC are suitable candidates for PRRT.[47] Van Essen *et al*.[48] treated nine PGL/PCC patients with a total administered activity ranging from 14800 to 29600 MBq (400–800 mCi) ^{177}Lu-octreotate. None of the patients achieved a complete response on tumour volume; however, a partial response or stable disease was achieved in, respectively, two and four patients. In a study by Imhof *et al*.[49], 11 patients with PCC and 28 patients with PGL were treated with ^{90}Y-DOTATOC therapy. Seven patients had a partial response after therapy.

Not all patients with malignant PGL/PCC are eligible for MIBG therapy, as it depends on whether the tumours exhibit adequate take up of the radiopharmaceutical after intravenous administration.[50,51] To establish whether a patient is a good candidate for either ^{131}I-MIBG therapy or PRRT, ^{123}I-/^{131}I- MIBG scintigraphy or ^{111}In-pentetreotide scintigraphy (SRS), respectively, has to be performed in advance. In patients with malignant PGL/PCC with poor ^{123}I-MIBG uptake, but good uptake with SRS, PRRT might be a good alternative treatment for ^{131}I-MIBG therapy.

Chemotherapy is another treatment option for patients with malignant PGL/PCC, but large clinical trials are lacking. Huang *et al*.[52] showed a complete response of tumour volume in 11% and a partial response in 44% of 18 patients treated with a combination chemotherapy of cyclophosphamide, vincristine and dacarbazine (CVD). The 5-year overall survival was <50% and did not differ between the patients whose tumours responded to therapy and those whose tumours did not respond. Nomura *et al*.[53] also did not find a better survival in patients treated with CVD chemotherapy compared with controls. An advantage of ^{131}I-MIBG therapy is that, unlike chemotherapy, it has a mild toxicity in general. In our review, haematologic toxicity was the most reported side effect of included studies, usually neutropenia and/or thrombocytopenia. Unfortunately, the timing at which these side effects were noted is missing in most cases. Sze *et al*.[54] recently published a study about the incidence of persistent haematological and renal toxic effects after long-term follow-up of ^{131}I-MIBG therapy in fourteen patients with PGL/PCC and 44 patients with NETs. Overall,

32 patients (26 patients with NET and six patients with PGL/PCC) developed new and/or sustained haematological effects. Five patients (four patients with NET and one PGL/PCC patient) were noted to have more significant consequences, including the development of myelodysplasia (two patients), acute myeloid leukaemia (two patients) or chronic myeloid leukaemia (one patient). Number of cycles received and cumulative dose were significantly higher in this subgroup compared with those who did not develop haematological malignancy in this cohort. Compared with our included studies, Sze *et al*.[54] noted a substantial increase in the number of haematological events, including haematological malignancies. Probably, this is because of the longer follow-up of their patients. The median follow-up from commencement of first ^{131}I-MIBG therapy was, respectively, 47·5 and 46·5 months for the patients with NET and patients with PGL/PCC. Therefore, it is important to realize that although in our study the haematologic toxicity was mild, with prolonged follow-up and probably also with higher doses severe adverse events may become evident.

More recently, studies assessing targeted therapies, such as sunitinib, have shown promising results in the treatment of malignant PGL/PCC.[55] Sunitinib is an oral tyrosine kinase inhibitor with antiangiogenic and antitumour activity. Currently, the published data are limited to only a few case reports,[55,56] but a single arm open-label phase II trial with sunitinib is currently underway with an estimated study completion date of December 2013 (clinicaltrials.gov).

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Chapter 7

Chemotherapy with cyclophosphamide, vincristine and dacarbazine for malignant paraganglioma and pheochromocytoma: systematic review and meta-analysis

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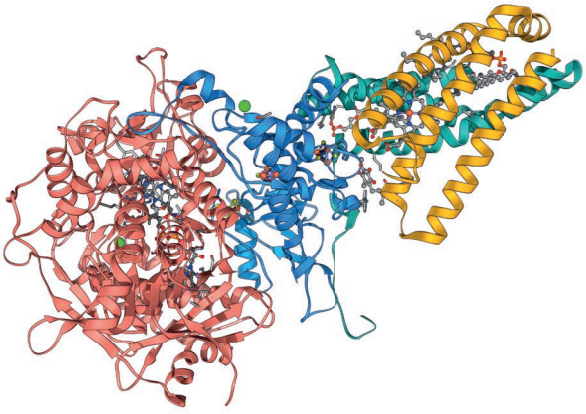

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Abstract

Background: Chemotherapy with cyclophosphamide, vincristine and dacarbazine (CVD) can be used for palliative treatment of malignant pheochromocytoma and paraganglioma. However, the precise effect of this chemotherapeutic regimen on tumor volume is unclear. The main objective of this study was to perform a systematic review and meta-analysis assessing the effect of chemotherapy with CVD on tumor volume in patients with malignant paraganglioma/pheochromocytoma.

Methods: A literature search was performed in October 2013 to identify potentially relevant studies. Main outcomes were the pooled percentages of complete response, partial response and stable disease after chemotherapy with CVD. A meta-analysis was performed with an exact likelihood approach using a logistic regression. Pooled percentages with 95% confidence intervals (CI) were reported.

Results: Four studies concerning a total of 50 patients with malignant paraganglioma/pheochromocytoma reported on treatment with a combination of CVD chemotherapy. A meta-analysis of the effect of chemotherapy on tumor volume showed pooled percentages of complete response, partial response and stable disease of, respectively, 4% (95% CI: 1%-15%), 37%(95% CI: 25%-51%) and 14% (95% CI: 7%-27%). Only two studies concerning a total of 35 patients assessed the response on catecholamine excess; pooled percentages for complete, partial and stable hormonal response were 14% (95% CI: 6%-30%), 40% (95% CI: 25%-57%) and 20% (95% CI: 10%-36%), respectively. Duration of response was also reported in only two studies with a median duration of response of 20 months and 40 months.

Conclusions: Data on the effects of a combination of CVD chemotherapy on malignant paraganglioma/pheochromocytoma suggest that a partial response concerning tumor volume can be achieved in about 37% of patients and a partial response on catecholamine excess in about 40% of patients. However, in the included studies, the protocol when to initiate treatment was not well described. Therefore, it can not be excluded that the reported effect of chemotherapy on tumor volume reflects the natural course of the disease, at least partially.

Introduction

Background

Paragangliomas (PGLs) are rare vascular, neuroendocrine tumors (NETs) of paraganglia. They derive from either sympathetic chromaffin tissue in adrenal and extraadrenal locations (sympathetic PGL or sPGL) or from parasympathetic tissue of the head and neck (HNPGL).[1] Approximately 80% of PGLs arise from the adrenal medulla and are referred to as pheochromocytoma (PCC).[1,2] Although the majority of PGLs are benign, there is a risk of malignant degeneration of 10% for PCC and 10-20% for sPGL.[3] Malignant disease is defined as the presence of metastatic lesions at sites where neuroendocrine tissue is normally absent.[4-7] The prognosis in malignant PGL/PCC is known to be poor and treatment remains basically palliative. The overall 5-year survival in patients with malignant PGL/PCC is less than 50%.[3,8,9] Patients with metastatic tumors also have high morbidity rates from excessive catecholamine secretion, hypertension and cardiovascular complications. Systemic treatment options include radionuclide therapy with ^{131}I-MIBG[10] or radiolabelled somatostatin analogues.[11] A recent meta-analysis on the effects of ^{131}I-MIBG therapy on malignant PGL/PCC suggests that stable disease concerning tumor volume and a partial hormonal response can be achieved in over 50% and 40% of patients respectively.[12]

Combination chemotherapy of cyclophosphamide, vincristine and dacarbazine (CVD) for the treatment of malignant PGL/PCC was introduced in 1985 by Keiser *et al.* [13] Three years later, Averbuch *et al.* presented a study in which 14 patients with malignant PGL/PCC were treated with this combination regimen of CVD. They reported a tumor response rate (complete and partial) of 57%.[14] Combination chemotherapy with CVD produced responses of 80% in children with advanced neuroblastoma, neuroendocrine tumors with similar clinical and biologic characteristics as PCC.[15]

At present, the precise effect of CVD chemotherapy for the treatment of malignant PGL/PCC is unclear. In 2007, Scholz *et al.* published a review on the current treatment of malignant PCC, including CVD chemotherapy.[16] They concluded that the CVD scheme seems to be effective at modest toxicity in a significant proportion of patients; however, remissions are rather short and are often followed by complete therapeutic failure after relapse.[16] A meta-analysis assessing this effect has never been performed.

Objective of the study

The aim of the present study was to perform a systematic review and meta-analysis of the effects of CVD chemotherapy on tumor volume in malignant PGL/PCC. Secondary objectives were to assess biochemical response (i.e. hormonal overproduction), overall survival, progression-free survival and toxicity.

Materials and methods

Eligibility criteria

Studies assessing the effect of the combination of chemotherapy with CVD on tumor volume of malignant PGL/PCC were eligible for inclusion. Malignant PGL/PCC was defined as the presence of metastases in non-neuroendocrine tissue distant to the primary tumor.[4-7] Studies concerning patients with non-malignant PGL/PCC according to this definition were excluded, e.g. locally invasive PGL/PCC without metastases, unless data for patients with metastatic PGL/PCC could be extracted separately.

The analysis aimed to assess the percentage of PGL/PCC-patients with tumor response after chemotherapy, with biochemical response (i.e. levels of catecholamines and/or their metabolites), overall survival, progression-free survival and toxicity as secondary outcomes. According to the "Response evaluation in solid tumors (RECIST) criteria" version 1.1, a partial treatment response is defined as "at least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters".[17] However, the RECIST criteria have not (yet) widely found their way in the field of PGLs. Therefore, it was decided not to restrict inclusion of studies to RECIST criteria only for tumor response.

To accurately assess response rates, only studies determining treatment response (tumor volume) by radiologic imaging were eligible for inclusion. Furthermore, only studies reporting a population of five or more PGL/PCC-patients were included, in order to avoid the inclusion of cases or case series which might be prone to selection and publication bias. In case of multiple studies describing the same cohort, the study which comprised the highest number of subjects and/or the longest duration of follow-up was included. Eligible studies were restricted to languages familiar to the authors (English, French, German and Dutch). When reported data were not sufficient for accurate data-extraction, we tried to contact the authors for clarification.

Search strategy

In October 2013 PubMed, MEDLINE, EMBASE, Web of Science, COCHRANE, CINAHL, Academic Search Premier and ScienceDirect were searched to identify potentially relevant studies (search strategy provided upon request). References of key articles were assessed for additional relevant articles.

Data extraction

All studies obtained from the search strategy were entered into reference manager software (Reference Manager version 12, Thomson Reuters, Philadelphia, PA) and were screened on title and abstract. Potentially relevant studies were retrieved for detailed assessment. For eligible studies, data were independently extracted by two reviewers (NN and GA).

Disagreements between reviewers were resolved by consensus, but when doubt remained, a third reviewer (EC) decided.

Risk of bias assessment

The present meta-analysis is based on observational studies. Risk of bias assessment was based on study components that potentially bias an association between the intervention under study (combination of chemotherapy with CVD) and the primary outcome (tumor volume). The following elements were assessed for all studies:

1. Risk of selection bias. Inclusion of consecutive exposed patients or a random sample of the inception cohort was considered a low risk of bias.
2. Adequacy of reporting of intervention (chemotherapy). When dose per cycle and number of cycles of chemotherapy were reported, this was considered adequate.
3. Adequacy of measurement of tumor volume. The effect of chemotherapy on tumor volume should have been measured by either MRI or CT scanning.
4. Adequate definition of tumor response. A prespecified definition of objective tumor response was considered adequate.
5. Adequacy of follow-up. Loss to follow-up < 5% was considered to represent a low risk of bias.

Statistical analysis

The main outcome of the present meta-analysis was the pooled percentage of PGL/PCC-patients with tumor response after CVD chemotherapy. The pooled percentage of PGL/PCC-patients with biochemical response after CVD chemotherapy was the secondary outcome. For all studies, the percentage of PGL/PCC-patients with tumor response was calculated as the number of PGL/PCC-patients with tumor response divided by the total number of PGL/PCC-patients treated with CVD chemotherapy. The same procedure was applied to the proportion of PGL/PCC-patients with biochemical response. For all percentages exact 95% confidence intervals (95% CI) were calculated.

Meta-analysis was performed using an exact likelihood approach. The method used was a logistic regression.[18] We considered a random-effects regression analysis by default, unless less than 5 studies contributed to a certain endpoint, because the between study variance can then not be assessed reliably. In such a case a fixed effect analysis was performed. For meta-analysis of proportions, the exact likelihood approach based on a binomial distribution has advantages compared to standard models based on normal distributions.[19] Firstly, estimates from a binomial model are less biased than estimates from models based on a normal approximation.[20] This is especially the case for proportions that are close to 0 or 1. Secondly, no assumptions are needed for the exact approximation when dealing with zero-cells. All analyses were performed with STATA 12.0 (Stata Corp, Texas, USA).

Results

Study selection

The initial search resulted in 459 unique records; 12 were selected for detailed assessment (Figure 1). After detailed assessment, 6 articles were excluded for the following reasons: outcome other than tumor response (n = 2), no original data (n = 1) and the number of PGL/PCC-patients did not exceed five (n = 3). Furthermore, 2 studies comprised a cohort also described in another publication; the studies with the smallest sample sizes were excluded.[14,21] No new articles were found in references of key articles. Finally, a total of 4 studies were included in the present analysis, all written in English.[22-25]

Study characteristics

Study characteristics are displayed in Table 1. Included studies were published from 2008 to 2013. All included studies were classified as cohort studies.[26] A total of 50 patients were included in this meta-analysis. The largest study contained 18 subjects. Mean age of included patients ranged from 34 to 47 years.

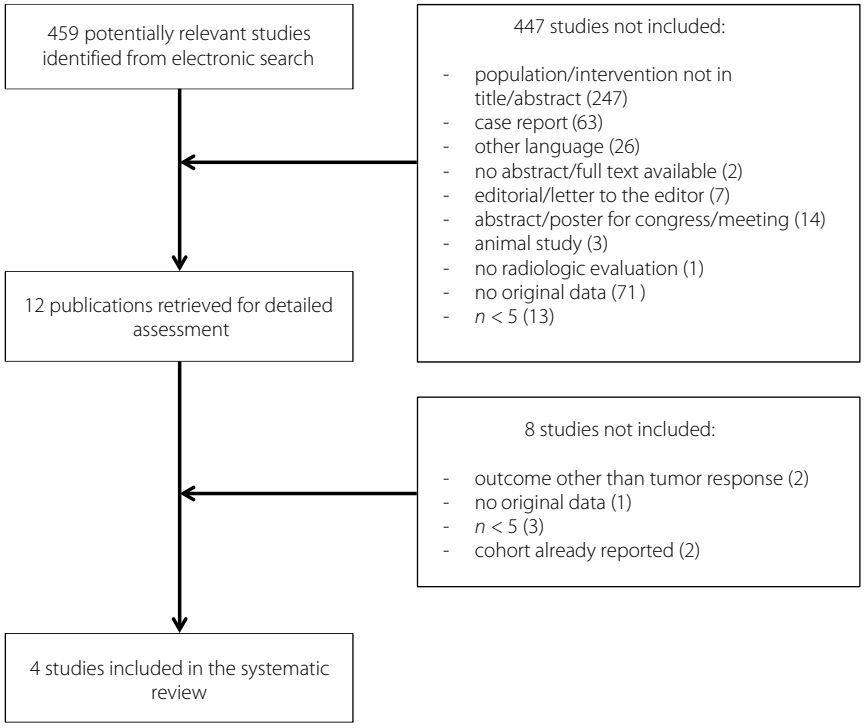

Figure 1. Flow-diagram of search strategy and study selection.

Risk of bias assessment

Summary characteristics of the risk of bias assessment are shown in Table 2. In all 4 studies included patients were explicitly described as consecutive exposed patients or as a random sample of the inception cohort. The intervention under study (i.e. CVD chemotherapy) was adequately described in 2 studies (50%). The effect of therapy on tumor volume was adequately measured (i.e. by CT and/or MRI) in all 4 studies. One study did not report prespecified definitions for assessment of tumor response. Actual loss to follow-up was reported in 3 of 4 studies (75%). In 2 of these 3 studies, loss to follow-up exceeded 5%.

Effect of CVD chemotherapy on tumor volume

Table 3 gives an overview of reported outcomes after CVD chemotherapy. To assess tumor response, one study used the RECIST criteria[24] and one study the RECIST 1.1 criteria.[25] One study used its own modified standard criteria[23] and one study did not report how tumor response was assessed.[22] Percentages of complete response after CVD chemotherapy ranged from 0% to 11%. For partial response, this was 24% to 50% and for stable disease 0% to 24%.

Results of the fixed effects meta-analysis are displayed in Figure 2. Pooled percentages of complete response, partial response and stable disease were 4% (95% CI: 1%-15%), 37% (95% CI: 25%-51%) and 14% (95% CI: 7%-27%), respectively.

Effect of CVD chemotherapy on catecholamine excess

Hormonal response was measured by only two studies. These two studies did not use standard criteria. The criteria used by the two studies are outlined in the appendix of Table 3. Percentages of complete response were 12% and 17%, partial response 24% and 55% and stable disease 17% and 24%.

The fixed effects meta-analysis resulted in pooled percentages of complete response, partial response and stable disease of, respectively, 14% (95% CI: 6%-30%), 40% (95% CI: 25%-57%) and 20% (95% CI: 10%-36%) (Figure 3).

Survival and side-effects of CVD chemotherapy

Information about survival and side-effects was only reported in three studies (Table 3). Side-effects comprised mainly gastrointestinal toxicity and myelosuppression.

Tabel 1. Study characteristics of included studies

First author (year)	Number of patients (*n*)	Mean age ± SEM (range)	Primary tumor localization (PGL/PCC) (*n*)	Prior treatment (*n*)	Dose of chemotherapy	Number of chemotherapy cycles (mean ± SEM)	Imaging modality	Tumormarkers	Treatment evaluation	Genetical analysis	Mean ± SEM duration of f-up (months) (range)	Loss of f-up (*n*+ reason)
Huang (2008)[23]	18	33.5 ± 3.4[a] (6-64)	PGL 9 PCC 9	Radiotherapy (6)	Cyclophosphamide 750 mg/m2[b], day 1 Vincristine 1.4 mg/m2, day 1 Dacarbazine 600 mg/m2, day 1 and 2 Every 21 to 28 days.	Patients whose tumors were scored as CR or PR: 27.4 ±5.5 cycles, median 23. Patients whose tumors did not respond: 8.75, median 5.5.	CT/^{131}I-MIBG	Urinary catecholamines, MN and VMA	Laboratory tests were repeated every 3-4 weeks throughout the treatment. Radiology and nuclear medicine studies were repeated every 6 to 16 weeks, if the original studies were abnormal	Presumed mutation: SDHB (3) Possible SDHB (2) SDHB or SDHD (4) SDHD confirmed (1)	Median potential follow-up 22 years	1, after 5 years, reason n.r.
Szalat (2011)[24]	5	39.2 ±5.5[c] (20-51)	PGL 1 PCC 4	Surgical resection (5) MIBG (1)	Cyclophosphamide, vincristine, dacarbazine[d]	Only reported for 2 patients; one had 6 courses and one 7 courses.	CT/MRI/^{18}F-FDG-PET/^{18}F-DOPA-PET/^{123}I-MIBG	Urinary E, NE, NMN, MN Blood CgA	Tumor response to therapy was based on clinical, biochemical, and imaging studies available in patients' files, n.s.	No systematic molecular genetic testing	n.r.	n.r.
Ayala-Ramirez (2012)[22]	10[e]	47.0 ± 3.9 (33-70)	PGL 5 PCC 5	Only reported for all patients. ^{131}I-MIBG (2)	Cyclophosphamide 600-750 mg/m2 Vincristine 1-2 mg/m2, Dacarbazine 750-1000 mg/m2.	Median 9.0	CT/MRI	n.r.	Responses were categorized as the best response during the first chemotherapy regimen	Unknown (7) SDHB and SDHD negative (1) SDHB/SDHC/SDHD negative (1) Ret negative (1)	n.r.	0

Tabel 1. Study characteristics of included studies *(Continued)*

First author (year)	Number of patients (*n*)	Mean age ± SEM (range)	Primary tumor localization (PGL/PCC) (*n*)	Prior treatment (*n*)	Dose of chemotherapy	Number of chemotherapy cycles (mean ± SEM)	Imaging modality	Tumormarkers	Treatment evaluation	Genetical analysis	Mean ± SEM duration of f-up (months) (range)	Loss of f-up (*n*+ reason)
Tanabe (2013)[25]	17[f]	At initiation of CVD 54.6±12.6 (mean±SD) At diagnosis of PCC/ PGL: 41.3±17.6	PGL 13 PCC 4	n.r.	Cyclophosphamide 750 mg/m2 on day 1, vincristine[g] 1.4mg/m2 on day 1, dacarbazine 600mg/m2 on day 1 and 2 every 21-28 days.	n.r.	CT/MRI/ ^{123}I-MIBG or ^{131}I-MIBG	Plasma and urinary NE, plasma CgA	Imaging modalities were performed before and after CVD chemotherapy, not further specified.	Germline mutation testing was not perfomed in any of the patients	median 60 months, range 12-192 months	6[f]

Abbreviations: *n.r.* not reported; *n.s.* not specified; *PCC* pheochromocytoma; *PGL* paraganglioma; *CT* computed tomography; *MRI* magnetic resonance imaging; *^{131}I-MIBG* ^{131}Iodine-metaiodobenzylguanidine; *^{123}Iodine-MIBG* ^{123}Iodine- metaiodobenzylguanidine; *^{18}F-FDG-PET* [18F]-fluorodeoxy-D-glucose positron emission tomography; *^{18}F-DOPA-PET* [18F]-fluorohydroxyphenylalanine positron emission tomography; *E* epinephrine; *NE* norepinephrine; *DA* dopamine; *MN* metanephrines; *NMN* normetanephrines; *VMA* vanillyl mandelic acid; *CgA* chromogranin A; *SDHB* succinate dehydrogenase subunit B gene; *SDHC* succinate dehydrogenase subunit C gene; *SDHC* subunit dehydrogenase subunit C gene; *SDHD* succinate dehydrogenase subunit D gene.

a Age at diagnosis.

b Initially, cyclophosphamide doses were escalated; however, if a patient had a nadir absolute neutrophil count less than $0.5x10^9$/L on 3 measurements or a platelet count less than $25x10^9$/L, the dose in the subsequent cycle was reduced 20% to 50%. The chemotherapy dose administered as a percentage of that planned was 80.0%, 74.7%, and 80.7% for cyclophosphamide, vincristine and dacarbazine respectively, in those whose tumors were scored as complete response or partial response, and 81.3%, 78.4% and 93.4% in those without a tumor response or with only minimal shrinkage.

c Age at first diagnosis.

d One patient received a therapeutic dose of mitotane to enhance the cytotoxic effect of chemotherapy.

e 54 patients received different chemotherapy regimens, 52 patients had their response status recorded, 10 patients received chemotherapy with cyclophosphamide, vincristine and dacarbazine. 19 patients received cyclophosphamide, vincristine, dacarbazine and doxorubicin, 12 patients received cyclophosphamide, doxorubicin and dacarbazine, 2 patients received cyclophosphamide, vincristine and doxorubicin, 1 patient received cyclophosphamide and doxorubicin, 1 patient CHOP, 1 patient Imatinib, 1 patient cisplatin and etoposide, 1 patient doxorubicin, vincristine and dacarbazine, 1 patient cyclophosphamide, vincristine and temozolomide, 1 patient carboplatin, etoposide and ifosfamide, 1 patient tamoxifen and 1 patient temozolomide, bevacizumab and sorafenib. Some data were only reported for the whole group of 52 patients.

f Originally, 23 patients received chemotherapy. Three patients were excluded from the analyses because of inadequate follow-up. CVD chemotherapy was discontinued in three cases because of poor general condition or adverse events (e.g., severe bone marrow suppression and liver dysfunction).

g The dosage of vincristine was limited to 2.0 mg/m2 body surface area/day according to an official instruction of the medicine in Japan. The treatment intervals were modified to 60-90 days in some patients after the 5th cycle for personal reasons (e.g. work schedule and economic factors).

Table 2. Risk of bias assessment of included studies

First author (Year of publication)	Consecutive patients or random sample of inception cohort	Determination of intervention adequately reported	Adequate measurement of tumor response	Adequate definition of tumor response	Number of patients lost to follow-up (%)
Huang (2008)[23]	Yes	Yes	Yes	Yes	1 (5.5%)
Szalat (2011)[24]	Yes	No[a]	Yes	Yes	n.r.
Ayala-Ramirez (2012)[22]	Yes	Yes	Yes	No[b]	0 (0%)
Tanabe (2013)[25]	Yes	No[c]	Yes	Yes	6 (26%)[d]

a Dose and number of cycles not reported.

b Response by tumor size was defined as any objective reduction in the size of the tumor on cross-sectional imaging studies during the first chemotherapy regimen. No definition of progressive disease.

c Number of cycles not reported.

d Three cases were excluded because of inadequate follow-up. CVD chemotherapy was discontinued in 3 cases because of poor general condition or adverse effects (e.g. severe bone marrow suppression and liver dysfunction).

Table 3. Outcomes of CVD chemotherapy

First author (year)	Number of patients (*n*)	Response on imaging			Response hormonal			Overall survival time/rate (confidence interval)	Progression-free survival time/rate (confidence interval)	Toxicity (*n*)	Toxicity grading system
		Complete response (*n*)	Partial response (*n*)	Stable disease (*n*)	Complete response (*n*)	Partial response (*n*)	Stable disease (*n*)				
Huang (2008)[23]	18	2 (11%)[a]	8 (44%)[b]	3 (17%)[c]	3[d] (17%)	10 (55%)	3[c] (17%)	Median survival for all patients from on-study date: 3.3 years. Median survival from date of diagnosis 6.5 years. Median survival from the landmark date: 3.8 years for patients whose tumors had a CR or PR to chemotherapy and 1.8 years for the rest[e].	Median duration of reponse: 20 months	Myelosuppression, peripheral neuropathy and gastrointestinal toxicity (n.s.) 4 episodes of hypotension in the first 3 cycles of treatment.	n.r.
Szalat (2011)[24]	5	0[f]	1 (25%)	0	n.r.	n.r.	n.r.	n.r.	n.r.	Only reported that one patient had severe myelotoxicity.	n.r.
Ayala-Ramirez (2012)[22]	10	0[g]	5 (50%)	0	n.r.	n.r.	n.r.	31.5 months for responders and 24.1 months for nonresponders[h]	n.r.	n.r.	n.r.

Table 3. Outcomes of CVD chemotherapy *(Continued)*

First author (year)	Number of patients (*n*)	Response on imaging			Response hormonal			Overall survival time/rate (confidence interval)	Progression-free survival time/rate (confidence interval)	Toxicity (*n*)	Toxicity grading system
		Complete response (*n*)	Partial response (*n*)	Stable disease (*n*)	Complete response (*n*)	Partial response (*n*)	Stable disease (*n*)				
Tanabe (2013)[25]	17	0[i]	4 (24%)[j]	4 (24%)	2[k] (12%)	4 (24%)[l]	4 (24%)	50% survival was 6 years in the patients with complete or partial biochemical and/or partial tumor response (n=8), and 4 years in the patients with no significant biochemical or tumor reponse (n=4), and 3 years in the patients with deterioration in biochemical and tumor responses (n=5)	Median 40 months, range 31-60 months	Grade 3 gastro-intestinal symptoms with discontinuation of CVD (1) Grade 2 leukopenia with discontinuation (1) Grade 2 liver dysfunction with discontinuation (1) Leukopenia grade 2 and liver dysfunction grade 1 lasting for up to 1 week after each CVD cycle Gastro-intestinal symptoms grade 2 and high fever grade 1 (transient and mild)	Adverse events were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0[m]

Abbreviations: *n.s.* not specified; *n.r.* not reported.

a Tumor response defined as: complete response (CR): complete regression of all clinical evidence of disease; partial response (PR): at least 50% reduction of all measurable tumor; minimal response (MR): at least 25% but not more than 50% reduction of all measurable tumor; no change (NC); progressive disease (PD): appearance of a new lesion or an increase of 25% of any measurable tumor.

b Also 3 patients (17%) with MR.

c Five patients reported with "no response", including patients with NC ánd PD. Because 14 of the 18 patients in this study were already reported by Averbuch *et al.* in 1988[14], and extracted data about NC and PD from those 14 patients were available, we extrapolated those data. For tumor reponse and for biochemical response, Averbuch *et al.*[14] reported 2 patients with NC and 1 patient with PD and Huang reported 5 patients with NC ánd PD. Therefore we extrapolated that there were 3 patients with NC and 2 patients with PD.

d Biochemical response defined as: CR: normal values; PR: at least a 50% reduction; MR: at least 25% but not more than 50% reduction; no change; PD: an increase of at least 25%, in all three measurements.
e Landmark date: 3 months after starting chemotherapy. Difference not statistically significant (p = 0.65).
f Response according to RECIST criteria.[31] Response only reported for 4 patients.
g Response by tumor size was defined as any objective reduction in the size of the tumor on cross-sectional imaging studies during the first chemotherapy regimen. Responses were categorized as the best response during the first chemotherapy regimen.
h Landmark time: 1 year from the start of chemotherapy. *P*-value 0.79.
i Tumor response according to RECIST 1.1 guidelines[17] and the standard criteria described by Averbuch *et al.*[14] Tumor response was classified as follows: CR: disappearance of all measurable tumor; PR: >50% tumor reduction; MR >25% but <50% tumor reduction; stable disease: no significant change in tumor; PD: the appearance of new lesion(s) or increased size >20% of total target lesions.
j Also 4 patients (24%) with MR.
k Biochemical response according to modified the criteria by Averbuch *et al.*[14] Biochemical response was classified as follows: CR: normalization of the biochemical tumor markers; PR: >50% reduction; MR: >25% and <50% reduction; NC: <25% reduction and <25% increase; PD: >25% increase of the biochemical tumor markers.
l Also 2 patients (12%) with MR.
m (http://evs.nci.nih.gov/ftp1/CTCAE/Documentation/CTCAE_Governance_2010-03-11.pdf)

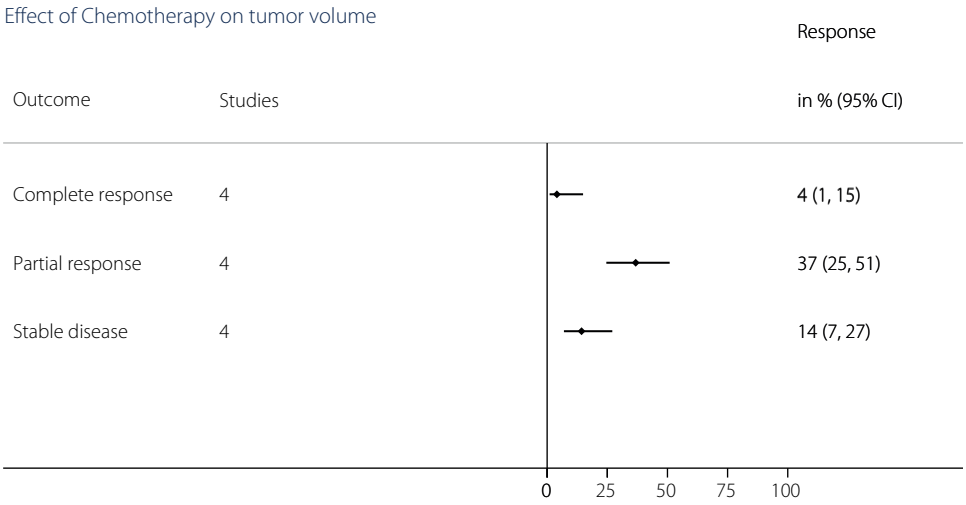

Figure 2. Effect of chemotherapy on tumor volume.

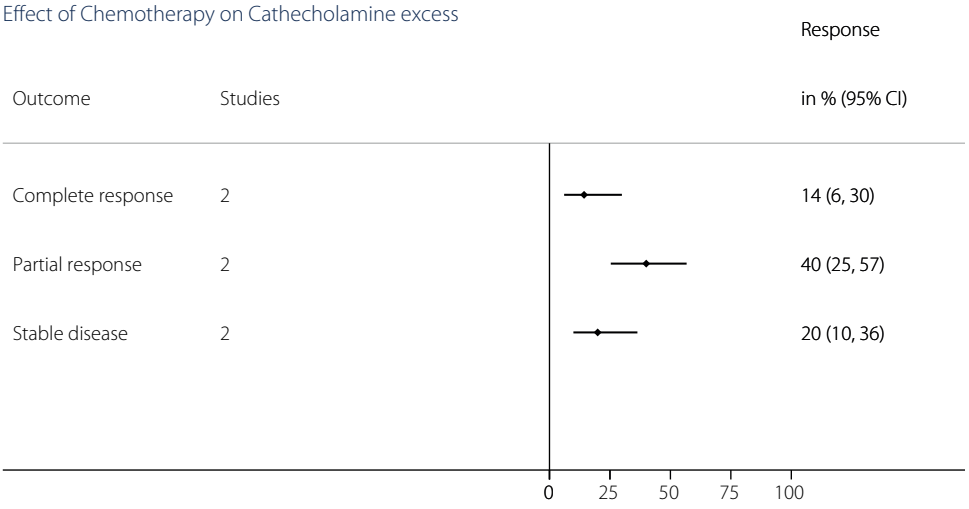

Figure 3. Effect of chemotherapy on catecholamine excess.

Discussion

The present systematic review and meta-analysis aimed to assess tumor response and hormonal overproduction of malignant PGL/PCC after CVD chemotherapy. Our meta-analysis showed that partial response on tumor volume following CVD chemotherapy could be achieved in about 37% of the patients and a partial hormonal response in about 40%. Complete response on tumor volume could be achieved in only 4% of patients. Toxicity leading to discontinuation of therapy was reported several times. With the results of this study, it is possible to inform both patients with malignant PGL/PCC and their treating physicians more adequately concerning the expected tumor response and the effect on survival after CVD chemotherapy.

In the included studies, the protocol when to initiate treatment was not well described. Only one study included patients with progressive metastatic disease; however, a definition of progression was not given.[22] The other three studies did not describe whether there was evidence of progressive disease. Hescot *et al.* recently published a study in which the natural history of patients with malignant PGL/PCC was assessed.[27] They found that half of the patients with metastatic PGL/PCC have stable disease at 1 year without any intervention. Therefore, they recommended a wait-and-see policy as first line management in asymptomatic patients with malignant PGL/PCC.[27] With regard to the results of our review, it can not be excluded that the reported effect of chemotherapy on tumor volume reflects the natural course of the disease, at least partially. CVD chemotherapy is a therapy regimen with potentially serious side effects like myelosuppression. Therefore it is important to realize that a wait-and-see policy might be a better option in asymptomatic patients.

Our meta-analysis showed that a partial response concerning catecholamine excess could be achieved in 40% of patients. This is a meaningful finding because reduced tumor function and, as a consequence, symptom palliation is an important treatment goal in patients with malignant PGL/PCC. Because quality of life was not an endpoint in the included studies, the question remains if the reduction in tumor function will lead to a better quality of life. Future studies with quality of life as an endpoint may probably point this out.

There are some limitations that should be taken into account when interpreting this meta-analysis. We could include only four studies with a total of 50 patients and only two studies reported effects of CVD therapy on catecholamine excess. This is, however, inevitable in view of the extremely low prevalence of malignant PGL/PCC. This means that results should be interpreted with caution as the reported effects may not be precise. In addition, because of the limited number of patients, a separate meta-analysis for PCC and PGL patients was not possible. Also, it would be of interest to assess responsiveness in different groups, for example men *vs* women, high *vs* low Ki67 index and presence or absence of a genetic syndrome, however, due to the low number of patients included in our meta-analysis, a

separate analysis for these different groups would lack statistical power.

We cannot rule out that the four cohorts listed might be different from each other concerning, for example progressiveness of the disease. Prior treatment regimen differed between 3 studies and was not reported in the other study. This difference in prior treatment regimen might be the result of more or less aggressive tumors in the included patients. This should be taken into account when interpreting these results.

Of the four included studies, two studies used RECIST and RECIST 1.1 criteria to assess tumor response. In one study, there was no adequate definition of tumor response[22] and another study used its own criteria.[23] Because of this heterogeneity, it is more difficult to compare the studies objectively. This may have contributed to differences in response rates. Also, when interpreting the data, it should be kept in mind that the analysis is based on four studies only, and that these four studies show clinical heterogeneity.

More recently, studies assessing targeted therapies, such as Sunitinib, have shown promising results in the treatment of malignant PGL.[28] Sunitinib is an oral tyrosine kinase inhibitor with antiangiogenic and antitumor activity. Currently, the published data are limited to only a few case reports and retrospective reports,[28-30] but a single arm open-label phase II trial with sunitinib is currently underway with an estimated study completion date of December 2013 (clinicaltrials.gov). Also, a first international, randomized, double blind, phase II, multicenter study has started in December 2011. This study aimed to determine the efficacy of Sunitinib on the progression-free survival at twelve months in subjects with progressive malignant PCC and PGL. The estimated study completion date of this study is December 2019 (clinicaltrials.gov).

In conclusion, with CVD chemotherapy a partial response concerning tumor volume can be achieved in about 37% of patients and a partial response on catecholamine excess in about 40% of patients with malignant PGL/PCC. However, the possibility remains that the reported effect on tumor volume reflects, at least partially, the natural course of the disease. Data are scarce and large clinical trials are lacking; therefore, more studies are needed to determine the precise effect of CVD chemotherapy.

Acknowledgements

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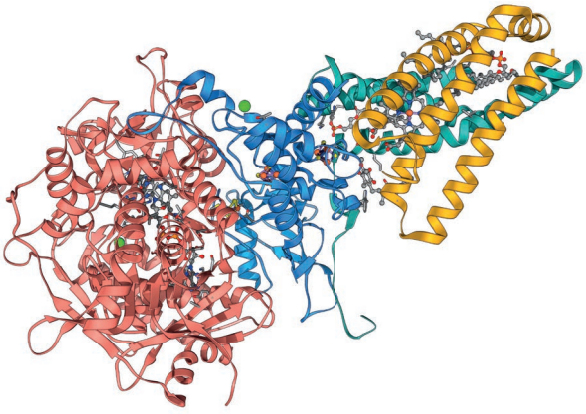
Chapter 8

Summary and conclusions



In the current thesis, we evaluated the clinical characteristics of *SDHx* mutation carriers, described genotype-phenotype correlations, assessed which (nonparaganglionic) tumors can also be linked to *SDHx* mutations and reviewed various treatment options for malignant pheochromocytoma (PCC)/paraganglioma (PGL).

Mutations in any one of the genes encoding succinate dehydrogenase (SDH) complex subunits or co-factors (*SDHA, SDHB, SDHC*, *SDHD*, and *SDHAF2*) can lead to formation of hereditary PGL syndromes. Mutations in *SDHB* and *SDHD* are generally the most common. In the Netherlands, the majority of the *SDH* mutation carriers harbor one specific mutation of *SDHD*, the c.274G>T, pAsp92Tyr.[1,2] *SDHB* mutations are less common, but the majority of *SDHB* mutation carriers also harbor known Dutch founder mutations, specifically the c423+1G>A mutation or the exon 3 deletion, c.201-4429_287-933del.[1] The reported penetrance of *SDHB* mutations (26-75%) is lower than the penetrance of *SDHD* or *SDHAF2* mutations (88-100% and 87-100%, respectively).[3-12] In **chapter 2**, an extended family with a founder exon 3 deletion in the *SDHB* gene was studied. From the 17 mutation carriers, 6 were clinically affected PGL patients. The calculated penetrance in this study was 9% at 50 years. The lower penetrance found in this study might reflect a clinical characteristic of this specific Dutch *SDHB* founder mutation, or the influence of a shared genetic or environmental modifier of penetrance in this family. However, it might also reflect an overestimation of *SDHB*-linked penetrance in the literature due to various forms of bias. In the literature, penetrance calculations are prone to overestimation because of the limited inclusion of unaffected mutation carriers and because the mutation carriers are identified via index patients. This might give a higher chance of selecting other mutation carriers with the disease (ascertainment bias). We included a relatively large number of unaffected mutation carriers and corrected for ascertainment bias. Also we excluded the index patient from the calculations. This resulted in reduced estimates of *SDHB*-linked penetrance and might be very important in the (genetic) counseling of *SDHB*-linked patients.

In **chapter 3**, we determined phenotypical characteristics of a large Dutch cohort of *SDHB* mutation carriers and assessed differences in clinical phenotypes related to specific *SDHB* mutations. We conducted a retrospective descriptive study in seven academic centers and included 196 *SDHB* mutation carriers. The study contained 65 (33.2%) index patients and 131 (66.8%) relatives. Fifty-four mutation carriers (27.6%) had one or multiple head and neck PGLs (HNPGLs), 4 (2.0%) had a PCC and 26 (13.3%) had one or more sympathetic PGLs. The figures for PCC en sPGL we found in our study were lower than that reported in previous studies that have assessed clinical characteristics in *SDHB* mutation carriers.[3,7,13,14] Because we included a large number of unaffected mutation carriers, ascertainment bias might play only a minor role. Furthermore, percentages mentioned in previous studies took into

account only disease-affected subjects. However, if we excluded all unaffected mutation carriers from our cohort, we still found lower figures for PCC and sPGL.
The frequency we found for HNPGLs (27.6%) was relatively high compared with other studies (6-31%)[3,7,13,14], and when we excluded all unaffected mutation carriers, our prevalence was as high as 65.1%. 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.
Only 15 patients (7.7%) developed a malignant PGL and 17 patients (8.7%) developed non-paraganglionic tumors, including 5 renal cell carcinomas (RCCs) and 2 gastric gastrointestinal stromal tumors (GIST).
Statistical analyses showed no significant differences in the number and location of HNPGLs, sPGLs or PCC, nor in the occurrence of malignant disease or other tumors between carriers of the two founder *SDHB* mutations (exon 3 deletion versus c.423+1G>A).
This study underlines the importance of the inclusion of unaffected identified carriers in studies that assess phenotypes of germline mutations. The results from this study are important to consider in the clinical management and genetic counseling of families with PCC/PGL syndromes. Including unaffected carriers provides a more accurate insight into the spectrum of disease.

In **chapter 4** we investigated which nonparaganglionic tumors belong to the SDH-associated tumor spectrum. If mutations occur in the *SDHA*, *SDHB*, *SDHC*, *SDHD*, or *SDHAF2* genes with corresponding loss of the wild-type allele or a second inactivating mutation, SDHB immunohistochemical staining will become negative.[15] This negative staining for SDHB is now a validated and highly sensitive marker for germline mutations of any of the SDH subunits and is a broadly accepted indication of pathogenicity of an *SDH* mutation.[16] In addition, SDHA immunohistochemistry is a proven marker for *SDHA* mutations, showing loss of immunoreactivity exclusively in *SDHA*-mutated tumors.[15] We analyzed 35 nonparaganglionic tumors from 26 *SDH* mutation carriers. Eight tumors showed negative staining for SDHB and positive staining for SDHA: a pancreatic neuroendocrine tumor (NET), a macroprolactinoma, two gastric GISTs, an abdominal ganglioneuroma, and three RCCs. A prolactinoma in a patient with a germline *SDHA* mutation was the only tumor immunonegative for both SDHA and SDHB. Sanger sequencing of this tumor revealed a somatic mutation (p.D38V) as a likely second hit leading to biallelic inactivation of SDHA. Our study strengthens the etiological association of *SDH* genes with pituitary neoplasia, renal

tumorigenesis, and gastric GISTs. Furthermore, our results indicate that pancreatic NET also falls within the SDH-related tumor spectrum. Our report was the first report of an association between a germline *SDHD* mutation and pancreatic NET. This finding might have potential implications for the surveillance of patients with a germline *SDHD* mutation, because in the existing surveillance protocol, abdominal imaging is only advised when there is evidence of catecholamine excess. It might be advisable to amend surveillance protocols, with the addition of standard abdominal imaging studies. However, the occurrence rate in our study was rare, and further studies are needed to definitely amend surveillance protocols.

Paragangliomas in the head and neck region can arise from the carotid body, vagal body, or jugulotympanic tissue (i.e. paraganglioma of the temporal bone). Due to their location in close proximity to important neurovascular structures, tumor growth may lead to serious morbidity and cranial nerve impairment. With removal of these tumors, branches of the carotid sinus nerves may not be spared. Bilateral carotid body tumor resection (bCBR) may thus result in arterial baroreflex dysfunction. In **chapter 5** we investigated the role of the baroreflex during sleep. We found that bCBR was associated with decreased low frequency power during sleep, suggesting impaired baroreflex function. The effect of sleep on heart rate was similar in bCBR patients and their matched controls, suggesting that the sleep-related heart rate decrease primarily results from non-baroreflex mediated pathways.

The risk of malignant transformation is 10% for PCC and 10-20% for extra-adrenal non-HNPGLs.[17] Treatment of malignant disease remains basically palliative. Radionuclide therapy using ^{131}I-MIBG has been investigated in several studies, however, with varying success rates. Because the precise effect of ^{131}I-MIBG therapy for the treatment of malignant PCC/PGL remained unclear, we performed a systematic review and meta-analysis. The results of this meta-analysis assessing the effects of ^{131}I-MIBG therapy on tumor volume in patients with malignant PCC/PGL are reported in **chapter 6**. We included 17 studies in our meta-analysis, with a total of 243 patients. We showed that stable disease following ^{131}I-MIBG therapy could be achieved in 52% of the patients and a partial hormonal response in 40%. Reported 5-year survival rates were 45% and 64% and mean progression-free survival times 23·1 and 28·5 months. The most frequent side effect was haematologic toxicity. In the included studies, the protocol when to initiate treatment differed widely. Many of the studies included patients irrespective of evidence of progressive disease. Therefore it might be possible that stable disease is not merely a therapy effect, but also a reflection of the natural course of the disease, with slow progression in a subset of patients.

Chemotherapy is another treatment option for patients with malignant PCC/PGL. Combination chemotherapy of cyclophosphamide, vincristine and dacarbazine (CVD) was

introduced in 1985 by Keiser *et al.*[18] A meta-analysis assessing the effect of CVD chemotherapy has never been performed. Therefore, in **chapter 7**, we performed a systematic review and meta-analysis addressing this effect. We included four studies concerning a total of 50 patients with malignant PCC/PGL. The meta-analysis of the effect of chemotherapy on tumor volume showed pooled percentages of complete response, partial response and stable disease of respectively 4% (95% CI: 1%-15%), 37% (95% CI: 25%-51%) and 14% (95% CI: 7%-27%). Only two studies concerning a total of 35 patients assessed the response on catecholamine excess; pooled percentages for complete, partial and stable hormonal response were 14% (95% CI: 6%-30%), 40% (95% CI: 25%-57%) and 20% (95% CI: 10%-36%), respectively. In the included studies, the protocol when to initiate treatment was not well described. Therefore it might be possible that the reported effect of chemotherapy on tumor volume reflects the natural course of the disease, at least partially.

Conclusions

The findings of this thesis can be summarized in the following conclusions:

1. The penetrance of the germline exon 3 *SDHB* mutation might be lower than previously described.
2. The inclusion of unaffected identified carriers in studies that assess phenotypes of germline mutations is very important to provide a more accurate insight into the spectrum and penetrance of disease.
3. The pancreatic NET is a new component of the SDH-related tumor spectrum. This might have potential implications for the surveillance of patients with a *SDHD* mutation, because at the moment abdominal imaging is not a standard part of the surveillance protocol.
4. After bilateral carotid body tumor resection, patients exhibit baroreflex dysfunction. Sleep-related heart rate changes are similar between bCBR patients and controls, suggesting that the effects of sleep on heart rate are predominantly generated through central, non-baroreflex mediated pathways.
5. In patients with malignant PCC/PGL, concerning tumor volume, stable disease following ^{131}I-MIBG therapy can be achieved in 52% of the patients and a partial hormonal response in 40%.
6. With CVD chemotherapy, a partial response concerning tumor volume can be achieved in about 37% of patients with malignant PCC/PGL and a partial response on catecholamine excess in about 40% of patients.

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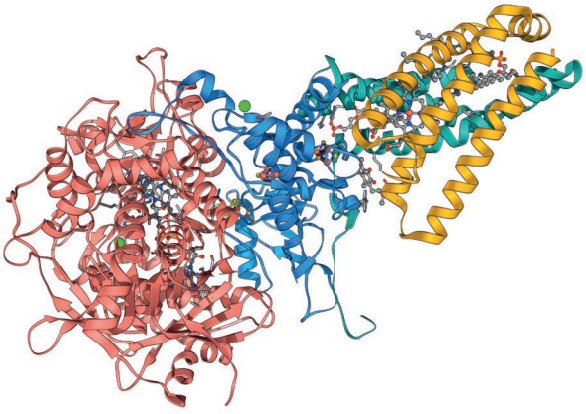
Chapter 9

Nederlandse samenvatting en conclusies



Paragangliomen (PGL) zijn zeldzame, vaatrijke neuroendocriene tumoren van het autonome zenuwstelsel. Ze zijn afkomstig van paraganglia; dit zijn orgaantjes die zijn ontstaan uit de embryonale neurale lijstcellen en die zijn gegroepeerd rondom het autonome zenuwstelsel. PGL kunnen voorkomen van het hoofd-halsgebied tot het bekken. PGL gelokaliseerd in het hoofd-hals gebied worden ook wel 'glomustumoren' genoemd. Als het PGL uitgaat van het bijniermerg, noemen we het een feochromocytoom (PCC). PGL buiten het hoofd-hals gebied maar niet in de bijnier, noemen we sympathische paragangliomen (sPGL). Deze kunnen voorkomen in de borstholte (thorax), in de buikholte (abdomen) en in het bekken (pelvis). Een groot deel van de PGL is erfelijk. Erfelijke PGL syndromen kunnen ontstaan door mutaties in het succinaat dehydrogenase (*SDH*) gen. Het *SDH* gen codeert voor SDH, een eiwitcomplex dat bestaat uit vier subunits (SDHA, SDHB, SDHC en SDHD) en wat zich bevindt op de binnenmembraan van mitochondriën. Het is een belangrijk enzym in zowel de elektronentransportketen (ademhalingsketen) als in de citroenzuurcyclus. In de elektronentransportketen staat het ook bekend als complex II. De vier SDH genen (*SDHA*, *SDHB*, *SDHC* en *SDHD*) coderen voor de vier subunits van complex II. Mutaties in één van deze vier subunits van het *SDH* gen veroorzaken dus de familiaire PGL syndromen. Hoewel alle vier de subunits (SDHA, SDHB, SDHC en SDHD) deel uitmaken van hetzelfde complex, geven mutaties in de verschillende genen andere fenotypische (klinische) effecten.
In dit proefschrift hebben we gekeken naar de klinische kenmerken van *SDHx* mutatiedragers, we beschrijven genotype-fenotype correlaties en we hebben onderzocht welke andere (niet-PGL) tumoren kunnen worden gerelateerd aan *SDHx* mutaties. Tevens geven we een overzicht van de verschillende behandelingsopties voor patiënten met een maligne (kwaadaardig) PCC/PGL.

De meest frequent voorkomende mutaties zijn mutaties in het *SDHB* en het *SDHD* gen. In Nederland heeft de meerderheid van de *SDH* mutatiedragers één specifieke mutatie in het *SDHD* gen, namelijk de c.274G>T, pAsp92Tyr mutatie.[1,2] *SDHB* mutaties komen minder frequent voor, maar de meerderheid van de *SDHB* mutatiedragers heeft ook één van de bekende Nederlandse founder mutaties, met name de c423+1G>A mutatie of the exon 3 deletie, c.201-4429_287-933del.[1] Bij *SDHx* mutatiedragers hoeft de ziekte niet altijd volledig tot uiting te komen. Het begrip dat weergeeft hoe vaak de ziekte tot uiting komt bij individuen met de (*SDHx*) genmutatie noemt met de penetrantie. De gerapporteerde penetrantie van *SDHB* mutaties (26-75%) is lager dan de penetrantie van *SDHD* mutaties (88-100%).[3-12] In **hoofdstuk 2** beschrijven wij een familie met een *SDHB* exon 3 deletie. Van de 17 mutatiedragers waren er 6 die klinisch waren aangedaan. De berekende penetrantie in deze studie was 9% op de leeftijd van 50 jaar. De lagere penetrantie die wij vonden in deze studie, kan een klinisch kenmerk zijn horend bij deze specifieke Nederlandse *SDHB* founder mutatie. Het kan ook het gevolg zijn van het vóórkomen van bepaalde genetische of

omgevingsfactoren in deze specifieke familie. Het kan echter ook zo zijn dat de penetrantie van *SDHB* mutaties in de bestaande literatuur wordt overschat door verschillende vormen van bias. In de bestaande literatuur zijn de penetrantie berekeningen onderhevig aan overschatting. Dit komt doordat er vaak weinig niet-aangedane mutatiedragers worden geïncludeerd en tevens doordat de mutatiedragers worden gevonden via index patiënten. Dit geeft een hogere kans op het selecteren van andere mutatiedragers met de ziekte ("ascertainment bias"). Wij includeerden een relatief hoog aantal niet-aangedane mutatiedragers en corrigeerden voor ascertainment bias. Ook werd de index patiënt uitgesloten van de penetrantie berekeningen. Dit resulteerde in een lagere penetrantie voor *SDHB* mutatiedragers, wat belangrijke consequenties kan hebben bij de (genetische) counseling van *SDHB* mutatiedragers.

In **hoofdstuk 3** beschrijven we de fenotypische kenmerken van een groot Nederlands cohort van *SDHB* mutatiedragers. Tevens evalueren we mogelijke verschillen in fenotype als gevolg van specifieke *SDHB* genmutaties. We hebben een retrospectieve studie verricht in zeven academische centra in Nederland en konden 196 *SDHB* mutatiedragers includeren. De bestudeerde studiepopulatie bestond uit 65 (33.2%) index patiënten en 131 (66.8%) familieleden van index patiënten. Vierenvijftig mutatiedragers (27.6%) ontwikkelden één of meerdere hoofd-hals PGLs (head and neck paragangliomas, HNPGL), vier patiënten (2.0%) een PCC en 26 (13.3%) één of meerdere sPGL. De aantallen die wij vonden voor PCC en sPGL zijn lager dan de getallen die worden gerapporteerd in eerder onderzoek naar de klinische kenmerken van *SDHB* mutatie dragers.[3,7,13,14] Doordat wij een hoog aantal niet-aangedane mutatiedragers hebben geïncludeerd, zal ascertainment bias mogelijk een kleinere rol spelen. In het merendeel van de eerder verrichte studies wordt namelijk een groter aantal index patiënten geïncludeerd en een kleiner aantal niet-aangedane mutatiedragers. Tevens zijn bij de berekeningen van de genoemde percentages in eerdere studies alleen klinisch aangedane mutatiedragers meegenomen. Indien wij echter alle niet-aangedane mutatiedragers uitsluiten in onze studie, vonden wij nog steeds lagere percentages voor het voorkomen van PCC en sPGL.
Het percentage mutatiedragers in onze studie met één of meerdere HNPGL (27.6%) was relatief hoog vergeleken met eerdere studies (6-31%).[3,7,13,14] Wanneer wij alle niet-aangedane mutatiedragers excluderen, wordt onze prevalentie zelfs 65.1%. Dit kan deels worden verklaard door het feit dat het percentage HNPGL patiënten met een positieve familie anamnese (dat wil zeggen niet-index HNPGL patiënten) in onze studie 29.6% is. De meerderheid van deze patiënten had geen symptomen en was dus nog niet onder de aandacht gekomen. Het genetisch onderzoek wat wordt aangeboden aan familieleden van mutatiedragers en tevens de gestructureerde follow-up protocollen in Nederland van patiënten met een *SDHB* mutatie zorgt voor de identificatie van een relatief hoog aantal

asymptomatische mutatiedragers, met of zonder tumoren. Dit zorgt voor een meer accurate representatie van het fenotype van *SDHB* mutatiedragers.
Vijftien patiënten (7.7%) ontwikkelden een maligne PGL en 17 patiënten (8.7%) ontwikkelden andere (niet-PGL) tumoren, inclusief vijf tumoren van de nier en twee gastro-intestinale stroma tumoren van de maag.
Een vergelijking van de fenotypen van de twee meest voorkomende *SDHB* genmutaties in ons cohort, de *SDHB* exon 3 deletie en de *SDHB* c.423+1G>A mutatie, toonde geen significante verschillen.
Deze studie geeft weer dat het belangrijk is niet-aangedane mutatiedragers te includeren in studies die het fenotype van genmutaties evalueren. De resultaten van deze studie zijn belangrijk bij de klinische behandeling en genetische counseling van families met erfelijke PCC/PGL syndromen. Het includeren van niet-aangedane mutatiedragers geeft een beter inzicht in het spectrum van de ziekte en de penetrantie van de mutatie.

In **hoofdstuk 4** hebben we onderzocht welke ándere (niet-PGL) tumoren behoren tot het *SDH*-geassocieerde tumor spectrum. Als er een mutatie optreedt in één van de *SDH* genen, met daarbij verlies van het wild-type allel of een tweede inactiverende mutatie, wordt de SDHB immunohistochemische kleuring negatief.[15] Een immunohistochemische kleuring is een kleuring die kijkt of het eiwit nog in de tumor aanwezig is. Deze negatieve eiwitexpressie in de tumor voor SDHB is een gevoelige marker voor het bestaan van een kiembaanmutatie in één van de SDH subunits. Het is wereldwijd geaccepteerd als indicator van pathogeniciteit (= ziekte veroorzakend) van een *SDH* mutatie.[16] Naast deze SDHB immunohistochemische kleuring is SDHA immunohistochemie een marker voor *SDHA* mutaties, waarbij er alleen in *SDHA*-gemuteerde tumoren verlies van SDHA immunohistochemie wordt gevonden.[15] Op basis van deze gegevens hebben wij 35 tumoren van 26 *SDH* mutatiedragers geëvalueerd. Acht tumoren toonden een negatieve SDHB kleuring en een positieve SDHA kleuring: een neuroendocriene tumor van de alvleesklier, een macroprolactinoom, twee gastrointestinale stroma tumoren van de maag, een abdominaal ganglioneuroom en drie nierceltumoren. Een prolactinoom van een patiënt met een kiembaan *SDHA* mutatie was de enige tumor die negatief was voor zowel SDHB als SDHA. Sanger sequencing van deze tumor toonde een somatische mutatie (p.D38V) als een mogelijke tweede *hit* leidend tot biallelische inactivatie van SDHA. Concluderend toont deze studie aan dat er een oorzakelijke associatie lijkt te bestaan tussen *SDH* genen en hypofyse tumoren, nierceltumoren en gastro-intestinale stromale tumoren van de maag. Tevens tonen onze resultaten dat de neuroendocriene tumor van de alvleesklier ook binnen het *SDH*-gerelateerde tumor spectrum valt. Onze studie was de eerste beschrijving van een associatie tussen een kiembaan *SDHD* mutatie en een neuroendocriene tumor van de alvleesklier. Deze bevinding kan potentiele implicaties hebben voor de surveillance van patiënten met een kiembaan *SDHD* mutatie. In het huidige

surveillance protocol wordt afbeeldend onderzoek van de buik namelijk alleen geadviseerd als er sprake is van hormonale overproductie van catecholamines. Misschien moeten de surveillance protocollen worden aangepast, met de toevoeging van standaard afbeeldend onderzoek van de buik. Wel was het vóórkomen van een neuroendocriene tumor van de alvleesklier in onze studie zeldzaam, waardoor meer onderzoek noodzakelijk is voordat we definitief de protocollen gaan aanpassen.

PGL in de hoofd-hals regio kunnen uitgaan van het glomus caroticum (ter hoogte van de carotisbifurcatie, de halsslagader), het glomus vagale (gelegen nabij de 10e hersenzenuw), of van het glomus jugulotympanicum (gelegen nabij het middenoor). Omdat deze HNPGL vaak gelegen zijn in de nabijheid van belangrijke neurovasculaire structuren, kan tumorgroei leiden tot ernstige morbiditeit en hersenzenuwuitval. Bij resectie van deze tumoren kunnen takken van de sinus caroticum niet altijd worden gespaard. De sinus caroticum is betrokken bij de regulatie van de hartfrequentie en de bloeddruk. Bilaterale verwijdering van glomus caroticum tumoren kan daarom resulteren in arteriële baroreflex dysfunctie, dat wil zeggen dat de regulatie van de bloeddruk niet meer adequaat is. In **hoofdstuk 5** hebben we onderzocht wat de rol is van de baroreflex tijdens slaap. We hebben hiertoe de polysomnografie (slaapregistratie) van negen patiënten die een bilaterale glomus caroticum resectie (bCBR) hadden ondergaan vergeleken met de polysomnografie van negen gezonde vrijwilligers (met gelijke leeftijd, geslacht en BMI). Wij vonden dat bCBR geassocieerd was met een verminderde baroreflex functie gedurende de slaap. Het effect van slaap op het hartritme was gelijk voor de bCBR patiënten en de controles. Dit suggereert dat de slaap-gerelateerde afname in hartfrequentie voornamelijk het gevolg is van niet-baroreflex gemedieerde pathways.

Het risico op maligne (kwaadaardige) ontaarding is voor een PCC 10% en voor een sPGL 10-20%.[17] De behandeling van gemetastaseerde (uitgezaaide) ziekte is met name palliatief. In verschillende studies is het effect van radionuclidentherapie met ^{131}I-MIBG onderzocht, met wisselende succespercentages. Radionuclidentherapie is behandeling met radioactieve stoffen. Omdat het exacte effect van ^{131}I-MIBG therapie voor de behandeling van maligne PCC/PGL onduidelijk was, hebben wij een systematische literatuurstudie en meta-analyse uitgevoerd. De resultaten van deze meta-analyse naar het effect van ^{131}I-MIBG therapie op tumor volume en hormonale parameters bij patiënten met een maligne PCC/PGL staan beschreven in **hoofdstuk 6**. Wij konden in onze meta-analyse 17 studies includeren met een totaal aantal van 243 patiënten. Wij vonden dat wat betreft tumorvolume stabiele ziekte kon worden bereikt in 52% van de patiënten en een partiële hormonale respons in 40%. De gerapporteerde 5-jaarsoverleving was 45% en 64% en de gemiddelde progressievrije overlevingstijd was 23.1 en 28.5 maanden. Hematologische toxiciteit werd het meest

frequent gemeld als bijwerking van ^{131}I-MIBG therapie. Het protocol wanneer de ^{131}I-MIBG therapie werd gestart verschilde echter evident in de geïncludeerde studies. Veel van de studies includeerden patiënten onafhankelijk van het feit of er sprake was van progressieve ziekte. Daarom is het mogelijk dat de stabiele ziekte niet alleen een therapie effect is, maar ook het natuurlijk beloop van de ziekte weergeeft, met langzame progressie in een deel van de patiënten.

Chemotherapie is een andere behandeloptie voor patiënten met maligne PCC/PGL. Combinatie chemotherapie met cyclofosfamide, vincristine en dacarbazine (CVD) werd in 1985 geïntroduceerd door Keiser *et al.*[18] Een meta-analyse naar het effect van CVD chemotherapie op tumorvolume en hormonale parameters is nog nooit verricht. Daarom hebben wij in **hoofdstuk 7** een systematische literatuurstudie en meta-analyse verricht naar dit effect. We konden vier studies includeren met een totaal aantal van 50 patiënten met maligne PCC/PGL. De meta-analyse naar het effect van chemotherapie op tumorvolume toonde gepoolde percentages van complete respons, partiële respons en stabiele ziekte van respectievelijk 4% (95% CI: 1%-15%), 37% (95% CI: 25%-51%) en 14% (95% CI: 7%-27%). Maar twee studies met in totaal 35 patiënten evalueerden het effect van CVD chemotherapie op hormonale parameters (catecholamine overproductie). Gepoolde percentages voor complete, partiële en stabiele hormonale respons waren respectievelijk 14% (95% CI: 6%-30%), 40% (95% CI: 25%-57%) en 20% (95% CI: 10%-36%). In de geïncludeerde studies werd het protocol wanneer de behandeling werd gestart niet goed beschreven. Het is daarom mogelijk dat het gerapporteerde effect van chemotherapie op tumorvolume het natuurlijk beloop van de ziekte weergeeft, in ieder geval partieel.

Conclusies

De bevindingen in dit proefschrift kunnen worden samengevat in de volgende conclusies:

1. De penetrantie van de *SDHB* exon 3 mutatie is mogelijk lager dan zoals beschreven in eerdere studies.
2. Het includeren van niet-aangedane mutatiedragers in studies die het fenotype van een kiembaanmutatie evalueren is erg belangrijk om een meer accuraat inzicht te krijgen in het spectrum en de penetrantie van de ziekte.
3. De neuroendocriene tumor van de alvleesklier kan worden gezien als een nieuwe component van het *SDH*-gerelateerde tumorspectrum. Dit kan potentieel van invloed zijn op het surveillance protocol van patiënten met een *SDHD* mutatie, omdat op dit moment beeldvorming van de buik hier geen deel van uitmaakt.

4. Bij patiënten die een bilaterale resectie van een glomus caroticum tumor hebben ondergaan, is er sprake van baroreflex dysfunctie. Er is geen verschil in de slaap-gerelateerde hartfrequentie veranderingen tussen bCBR patiënten en controles. Dit suggereert dat de effecten van slaap op het hartritme met name worden gegenereerd door centrale, niet-baroreflex gemedieerde pathways.
5. Bij patiënten met maligne PCC/PGL kan, wat betreft tumorvolume, stabiele ziekte na ^{131}I-MIBG therapie worden bereikt in 52% van de patiënten en een partiële hormonale respons in 40%.
6. Met CVD chemotherapie kan, wat betreft tumorvolume, een partiële respons worden bereikt in 37% van de patiënten met maligne PCC/PGL. Een partiële hormonale respons kan worden bereikt in 40% van de patiënten.

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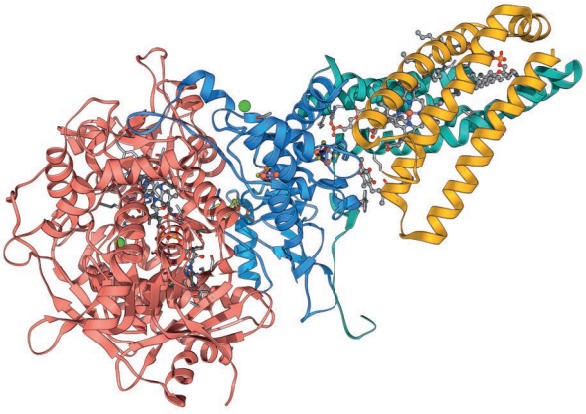
Curriculum Vitae



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Haar promotieonderzoek bij Dr. E.P.M. van der Kleij-Corssmit werd gestart in januari 2013 tijdens de opleiding tot internist-endocrinoloog, resulterend in dit proefschrift.

Na het afronden van haar opleiding in januari 2014 heeft Nienke gewerkt in het Havenziekenhuis in Rotterdam en vervolgens in het Amphia ziekenhuis. Sinds januari 2015 is zij werkzaam als internist-endocrinoloog in het IJsselland ziekenhuis in Capelle aan den IJssel.

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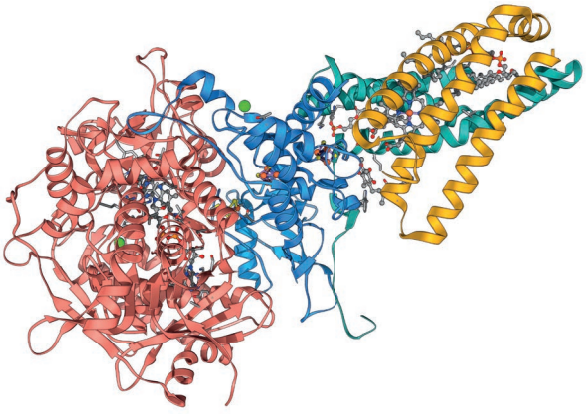

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