The handle http://hdl.handle.net/1887/19332 holds various files of this Leiden University dissertation.

**Author:** Duinen, Nicolette van  
**Title:** Hereditary paragangliomas : clinical studies  
**Issue Date:** 2012-03-07
Chapter 8
General discussion and summary
General summary and Discussion

Contents

I. Introduction

II. High prevalence of founder mutations of the succinate dehydrogenase genes in the Netherlands.

III. Biochemical screening of patients with head and neck paragangliomas.

IV. Chromogranin A as a tumor marker in patients with head and neck paragangliomas.

V. Pheochromocytomas detected by biochemical screening in predisposed subjects have a different clinical presentation compared to patients detected by signs and symptoms.

VI. Carotid body paragangliomas and sleep related complaints.

VII. Summary

I. Introduction

In recent years the multidisciplinary approach of the Leiden University Medical Center (LUMC) towards patients with head and neck paragangliomas (HNPGL) has been extended. All patients with HNPGL who consulted the departments of Otorhinolaryngology, Endocrinology and/or Clinical Genetics were screened for catecholamine excess according to a routine, structured protocol. The aim of inclusion of all consecutive HNPGL patients in the LUMC was to further specify the clinical and biochemical characteristics of these patients. Initial screening consisted of measurement of 24-hour urinary excretion of catecholamines and their O-methylated degradation products in duplicate, which was repeated with intervals of 2 years, if initial biochemical screening was negative. In patients with excessive catecholamine excretion, imaging studies with $^{123}$I-MIBG scintigraphy and whole body MRI and/or CT were performed to exclude additional intra- or extra-adrenal non HNPGL.

In this thesis we describe the genetic, biochemical and clinical characteristics of these HNPGL patients. In addition to screening of urinary catecholamine excretion rates, we measured plasma metanephrine levels and compared test sensitivity of these plasma levels with urinary excretion rates of catecholamines. Furthermore, we assessed whether plasma chromogranin A levels has a role as tumor marker in HNPGL patients. A previous study, conducted by B. Havekes et al (unpublished data), described an association between glomus caroticum tumors and subjective sleep related complaints. To study these
complaints in more detail we screened patients with bilateral carotid body paragangliomas for sleep disordered breathing.

II. High prevalence of founder mutations of the succinate dehydrogenase genes in the Netherlands

The hereditary paraganglioma syndrome is caused by mutations in the succinate dehydrogenase (SDH) gene. The SDH gene family (SDHA, SDHB, SDHC and SDHD) encodes the four subunits of complex II of the mitochondrial electron transport chain. SDH contributes to energy metabolism as a component of the tricarboxylic acid cycle, converting succinate to fumarate, and by serving as a source of electrons for mitochondrial respiration, as complex II of the electron transport chain. Because ~30% of patients with apparently sporadic HNPGL are affected by a SDHx mutation, molecular genetic screening should be performed in all patients with HNPGL. The LUMC is a dedicated referral laboratory for paragangliomas in the Netherlands. As almost all DNA samples of Dutch paraganglioma patients are tested in the LUMC, mutation frequency analysis of these patients represents the actual prevalence of SDH gene mutations in the Netherlands. Analysis of 1045 paraganglioma and pheochromocytoma patients and their relatives indicated that the large majority of mutations in SDH subunits or co-factors involve SDHD, followed by SDHB and SDHAF2 mutations, whereas SDHC mutations are extremely rare. The majority of SDH mutation carriers in the Netherlands harbour one specific mutation of SDHD, p.Asp92Tyr (chapter 2). This mutation alone accounts for 69% of all mutations in genes encoding subunits of the SDH complex. Several very large families residing in the western part of the Netherlands have been linked to this mutation and a strong founder effect has been demonstrated (1). Compared to the high prevalence of SDHD mutations in The Netherlands (87%), SDHB mutations are strikingly less common (6%). The majority of SDHB mutation carriers also have one of the two known Dutch founder mutations: c.423+1G>A or c.201-4429_287-933del (2;3).

These results differ from international studies in other countries, which show either a two-fold higher frequency of SDH mutations carriers (4), a two-fold higher frequency of SDHD-related patients (5), or approximately equal numbers (6;7), but none showed the 14-fold higher frequency found in The Netherlands. These differences in mutation prevalences can be explained by the unusual social and demographic history of our country, factors that might have contributed to the prevalence of a notable number of founder mutations in other genes (8). The Netherlands showed high levels of endogamy, marriage within groups, these groups defined by religious, geographic, or linguistic isolation, or a combination of these factors (9). The isolation of communities due to religious barriers to intermarriage was perhaps the most enduring factor, lasting well into the twentieth century, but occupational and geographic isolation were also important factors. These obstacles to intermarriage led to the creation of many genetically isolated populations. Such populations facilitate the proliferation of founder mutations and the well-known Dutch SDHD founder mutation, p.Asp92Tyr, shows a specific geographic focus even today (10). The region harboring the largest p.Asp92Tyr related pedigree also remained Catholic despite the overwhelming
dominance of the Protestant religion in surrounding areas. The prevalence of this specific SDHD mutation is therefore most likely attributable to endogamy, this effect perhaps amplified by limited migration, and rapid population growth in the 20th century (11). The absence of a similar effect in the case of SDHB mutations is probably due to simple chance.

**Clinical implications:** As mutations of SDHB, SDHC, SDHD and SDHAF2 each result in distinct hereditary paraganglioma syndromes, with differing modes of inheritance, penetrance, risk of pheochromocytoma, and risk of malignant paraganglioma, the identification of the affected gene is essential in providing effective genetic counselling to the individual paraganglioma patient. To date, several algorithms for prioritizing the order of gene-specific mutation testing in paraganglioma patients have been proposed, with the objectives of minimizing mutation screening and cost reduction (12;13). While these algorithms represent a very useful departure point for genetic analysis, it is doubtful whether the effectiveness and outcome of such algorithms is universally applicable, as the *a priori* chance of finding a mutation in a specific gene differs from country to country.

Knowledge of these regional differences in the prevalence of mutations will facilitate the tailoring of genetic screening protocols to local circumstances.

### III. Biochemical screening of patients with head and neck paragangliomas

Head and neck paragangliomas have the ability to produce and secrete catecholamines. Erickson *et al.* reported that a small proportion (4%) of benign HNPGLs were hyperfunctional (14). In 2005 van Houtum *et al.* documented that the prevalence of catecholamine excess in our SDHD-linked HNPGL patients was much higher than previously appreciated (15). At the time of that study, 15 of 40 consecutive patients (37.5%) had elevated levels of urinary catecholamine excretion. Pheochromocytomas or extra-adrenal paragangliomas were identified in 8 of these 15 patients (20%), indicating that HNPGL may be responsible for excess catecholamine secretion in about 17.5% of the cases included in their study. Importantly, they found no relationship between the levels of catecholamine excess and the complaints generally attributed to catecholamine excess. In chapter 3 we describe the results of biochemical screening in 136 patients with HNPGLs. Thirty-nine (29%) of the 136 included patients had excessive urinary excretion rates of catecholamines. The majority of the patients with a biochemical active tumor (31 of 136 patients, 23%) had increased urinary excretion of 3-methoxytyramine (3MT), associated with increased dopamine excretion. Patients with 3MT excess had significantly more complaints of palpitations (p<0.01), diaphoresis (p=0.03), collapse (p<0.05) and a higher pulse rate (p<0.01). Increased excretion of 3MT was not associated with particular types of HNPGL or genotypes.

It was unknown whether plasma catecholamine levels, including 3MT, were more sensitive parameters of biochemical activity of HNPGL than urinary excretion rates. For the diagnosis of pheochromocytoma, the measurement of plasma free metanephrine levels is the optimal biochemical test with the highest sensitivity and specificity (16). Therefore, in chapter 4 we studied whether plasma free metanephrines and 3MT levels are more sensitive tests to detect biochemical activity of HNPGL than urinary excretion rates of...
catecholamines and 3MT. We screened 124 HNPGL patients for catecholamine excess by measurement of 24-hr urinary excretion rates of (nor)metanephrine, (nor)epinephrine, vanillylmandelic acid (VMA), dopamine and 3MT and plasma levels of (nor)metanephrine and 3MT. Plasma 3MT levels were increased in 35 of the 124 patients (28%), whereas 24-h urinary excretion rates of 3MT were increased in 30 patients (24%) (p=0.13). Plasma combined metanephrine levels (NMN, MN, 3MT) were increased in 41 patients (33%), whereas 24-h urinary excretion rates of combined metanephrines were increased in 33 patients (27%, p£0.05). The combined assessment of plasma concentrations of free metanephrines and 3MT indicate a higher number of biochemically active HNPGL than the measurement of 24 h urinary excretion rates of combined metanephrines and 3MT. In addition, these data indicate that in HNPGL patients urinary excretion rates of deconjugated 3MT and plasma free 3MT levels do not indentify significantly different numbers of subjects with biochemical active HNPGL.

Clinical implications: Our results indicate that the number of patients with biochemical active HNPGLs is much higher than hitherto appreciated in studies that did not include the measurement of 3MT. We found that only part of the HNPGL patients with increased urinary excretion of 3MT also had increased urinary dopamine excretion. Therefore, urinary 3MT excretion is more sensitive in discovering dopamine-producing HNPGL than urinary dopamine excretion. We observed that the clinical manifestations in patients with increased 3MT excretion were different compared to those in patients with normal excretion of 3MT. Test sensitivity of plasma 3MT measurement equals the measurement of urinary deconjugated 3MT excretion. The combined assessment of plasma metanephrine levels (NMN, MN, 3MT) indicates a higher number of biochemical active tumors than the measurement of 24 hour urinary excretion rates of combined metanephrines and catecholamines.

IV. Chromogranin A as a tumor marker in patients with HNPGL

Although HNPGL have the ability to produce and secrete catecholamines (17;18), we recently demonstrated that only 29% of patients with HNPGL have evidence of increased urinary excretion of catecholamines and/or their metabolites (19). Consequently, the majority of these patients have biochemical silent tumors and the clinical characteristics of these HNPGL can be evaluated only by imaging techniques. Chromogranin A (CgA) is a secretory protein from neuroendocrine cells that mediates chromaffin granule biogenesis, necessary for catecholamine storage (20;21). CgA is secreted from neurosecretory vesicles, along with catecholamines (22). Plasma CgA is a useful tumor marker in patients with pheochromocytomas (23-29). Increased plasma levels of f chromogranin A have been found in some patients with HNPGL, indicating the presence of secretory granules(30). In chapter 5 we present the results of the measurement of plasma chromogranin A (CgA) levels in patients with hereditary HNPGLs. Plasma CgA levels were increased in a minority of HNPGL patients, only 16% of all patients had increased plasma CgA levels. In the patients with biochemically inactive tumors, 15% had increased plasma CgA levels.
Therefore the practical implications of the measurement of CgA in HNPGL patients are limited. Interestingly, urinary excretion rates of noradrenaline and normetanephrine were positively related with plasma CgA levels. However, we found no relation between the urinary excretion rates of 3MT and dopamine and plasma CgA levels. This indicates that increased plasma CgA levels are associated with increased noradrenergic activity, but not with increased dopaminergic activity. This might indicate that the secretion of noradrenaline differs from the secretion of dopamine by HNPGL. However, at present the precise role of CgA in the monoamine sorting process in the chromaffin cells is still unclear and additional studies are required to elucidate the role of CgA in the sorting and transport of dopamine into granule vesicles.

V. Pheochromocytomas detected by biochemical screening in predisposed subjects have a different clinical presentation compared to patients detected by signs and symptoms

Pheochromocytomas are rare neuroendocrine tumors derived from chromaffin tissue within the adrenal medulla (31). Pheochromocytomas can be caused by germline mutations in the von Hippel-Lindau gene (VHL), the RET gene (leading to MEN2), the neurofibromatosis type I gene (NF1) or one of the SDH genes encoding for subunits B, D and C of mitochondrial succinate dehydrogenase (32-38). Because of this hereditary predisposition, patients with germline mutations in the VHL, RET, NF1 and SDHx genes are screened for the development of pheochromocytomas. In chapter 6 we compared the differences in presentation, treatment and long-term follow-up of patients with pheochromocytomas detected by biochemical screening in hereditary syndromes predisposing for pheochromocytomas and patients with sporadic pheochromocytomas. Patients with hereditary tumors presented in an earlier stage of tumor formation with smaller tumors. The levels of catecholamine secretion correlated with tumor diameters. Therefore, patients with hereditary tumors had lower urinary excretion rates of catecholamines which resulted in a lower prevalence of signs and symptoms compared to the patients with sporadic tumors. Despite these differences in biochemical activity and the sizes of the pheochromocytomas there were no differences between both groups in peri-operative complications. This was probably related to careful pre- and (peri-) operative care with careful titration of alpha- and beta blocking drugs. Long term follow up revealed additional manifestations of disease in both groups of patients. In the patients with a documented hereditary predisposition several patients developed an additional pheochromocytoma in the contralateral adrenal gland, especially in the case of MEN 2A syndrome (39). In the sporadic group there were several patients with malignant pheochromocytomas. Therefore, long term follow up seems to be warranted in all pheochromocytoma patients, irrespective of initial presentation.

Clinical implications: Patients at risk for developing a pheochromocytoma should be regularly screened. The diagnostic test of choice is the measurement of fractionated plasma and/or urinary metanephrines (40). Patients with a SDHx mutation, MEN2 disease or Von Hippel-Lindau disease are advised to be screened for pheochromocytoma every 1-2 years,
depending on the mutation. In case of increased plasma/urinary catecholamines or their metabolites, additional radiological investigation should be performed to identify the culprit lesion (41-44). The prevalence of pheochromocytoma is quite low in patients with Neurofibromatosis and therefore screening is not recommended in all patients, but it may be justified in those patients with neurofibromatosis with hypertension, or in those patients who will undergo provocative interventions, such as surgery or pregnancy (45). The age of initial screening is determined by the specific gene mutation (46).

VI. Carotid body paragangliomas and sleep related complaints

Quality of life (QoL) studies performed in patients with head and neck paragangliomas reported that HNPGL patients frequently reported fatigue, reduced exercise tolerance and impaired sleep associated with the presence of carotid body glomus tumors (47). To elucidate the pathophysiology between sleep related complaints in patients with carotid body glomus tumors we studied 9 patients with bilateral carotid body tumors (bCBT) and 9 patients with bilateral carotid body resection for sleep disordered breathing by polysomnography (chapter 7). There was a high prevalence of sleep disordered breathing in patients with bilateral carotid body paragangliomas, but not in patients with bilateral resection of carotid body paragangliomas. Moreover, bCBT patients reported an impaired quality of life and a reduced daytime activity level compared to healthy controls. Sleep disordered breathing was associated with increased carotid body output, evidenced by an increased peripheral chemoresponsiveness. The question arises whether the increased chemoresponsiveness in the bCBT patients is associated with mutations in the succinate dehydrogenase gene (SDH). Piruat et al. have tested whether mice with a partial SDHD deficit have altered carotid body function. They found that the loss of an SDHD allele results in an abnormal enhancement of resting CB activity. This CB overactivity was linked to glomus cell hypertrophy and hyperplasia (48). Therefore, the increased peripheral chemoresponsiveness in the bCBT patients might be the result of hyperplasia of carotid body cells, indicating that tumor formation in the carotid bodies leads to gain of function rather than loss of function. Intermittent hypoxia can lead to the development of systemic hypertension, heart failure, myocardial infarction and stroke (49). Therefore, it is important to treat sleep disordered breathing. The optimal treatment strategy for bCBT patients with severe sleep apnea remains to be elucidated.
VIII. Summary

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

1. In the Netherlands, the majority of SDHx mutation carriers harbor a mutation in the SDHD gene, followed by SDHB and SDHAF2 gene mutations, whereas SDHC mutations are extremely rare.

2. Almost 90% of all SDH-related paraganglioma and pheochromocytoma cases in the Netherlands can be attributed to only 6 founder mutations.

3. Twenty-nine percent of patients with head and neck paragangliomas have evidence of a biochemical active tumor. The majority of patients with biochemical active head and neck paragangliomas have increased urinary excretion of 3-methoxytyramine, a metabolite of dopamine.

4. Test sensitivity of plasma 3MT measurement equals the measurement of urinary deconjugated 3MT excretion. The combined assessment of plasma metanephrine levels (NMN, MN, 3MT) indicates a higher number of biochemical active tumors than the measurement of 24h urinary excretion rates of combined metanephrines and catecholamines.

5. Only a minority of HNPGL patients have increased plasma chromogranin A levels. Therefore, the practical implications of the measurement of plasma CgA levels are limited in HNPGL patients.

6. Increased plasma CgA levels are associated with increased noradrenergic activity, but not with increased dopaminergic activity. This might indicate that the secretion of noradrenaline differs from the secretion of dopamine in HNPGL.

7. Patients screened for pheochromocytoma, because of a hereditary predisposition, present with less signs and symptoms, lower urinary excretion rates of catecholamines, and smaller tumors than patients presenting with symptomatic pheochromocytomas. Despite these differences in biochemical activity and the sizes of pheochromocytomas there is no difference between patients in perioperative complications.

8. Patients with bilateral carotid body tumors are at risk for developing sleep disordered breathing. Sleep disordered breathing is associated with increased carotid body output, which is reflected by increased chemosensitivity.