Chapter 1

General Introduction

Adapted from:

Update on Pediatric Pheochromocytoma


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1 General introduction

1.1 The Autonomic Nervous System and the Paraganglion System

The autonomic nervous system is necessary for maintaining homeostasis and its regulation is involuntary. In general, the autonomic nervous system consists of a sympathetic and parasympathetic part that most often have complementary effects on organ function. The sympathetic division is often described as triggering a fight or flight response, with for example an increase in blood pressure and heart rate as a consequence. The peripheral autonomic nervous system can be divided in sensory neurons that carry information to the central nervous system and motor neurons which subsequently carry these signals to their effector organs like muscles or glands. These autonomic motor neurons consist of two-neuron chains, pre-ganglionic and post-ganglionic motor neurons, of which the latter’s cell bodies reside in so-called autonomic ganglia in between. In both sympathetic and parasympathetic pre-ganglionic and in parasympathetic postganglionic neurons, acetylcholine is used as a neurotransmitter. The sympathetic postganglionic system uses catecholamines (epinephrine, norepinephrine) to exert its effect on the target organs. Epinephrine and norepinephrine exert their effects through α- and β-adrenoceptors on various organs with different effects on several separate receptor subtypes (1).

Strongly associated with this autonomic nervous system are the widely dispersed cell clusters of neuroectodermal origin, which are called paraganglia (singular: paraganglion). During embryologic development these neural crest derived cells migrate along innervating nerves and vasculature towards their final location (Figure 1)(2). The term paraganglia is used because numerous paraganglia are located in the direct proximity of the sympathetic ganglia chain. In general, paraganglia in the head-and-neck region are associated with the parasympathetic system, whereas other paraganglia are related to the sympathetic nervous system. In the medulla of the adrenal gland the neural crest cells eventually form the chromaffin cells that are able to produce and secrete catecholamines, thus functioning as an important neuro-endocrine organ. Most of the paraganglion system outside the adrenals regresses as the adrenal gland matures after birth. In the head-and-neck region the most prominent paraganglionic structure is the carotid body that is located at the carotid artery bifurcation. The first anatomical description of the most prominent head-and-neck paraganglion dates back to the 18th century. Albrecht von Haller (1708-1777) was a Swiss scientist who defended his PhD thesis at the Leiden University in 1727 (promotor: H. Boerhaave) and discovered the carotid body in 1742 (3). It functions both as a peripheral chemoreceptor that registers oxygen tension and has a role as a baroreceptor, providing the autonomic nervous system with necessary feedback considering oxygen levels and blood pressure.

Because of cellular migration during embryologic stages and subsequent involution of a considerable proportion of the paraganglia after birth, the occasional
development of paraganglionic tissue at unusual locations is comprehensible. Furthermore, because of their close relation to the autonomic nervous system,

**Figure 1: Distribution of the paraganglion system in a newborn child**

Adapted from: J.C. Jansen, P.B. Douwes Dekker. Courtesy of AFIP, Tumors of the extra-adrenal paraganglion system (including chemoreceptors), Washington DC, Armed Forces Institute of Pathology, 1974

these cells have the capability to produce and secrete catecholamines, which can result in particular clinical syndromes that will be explained further in this thesis.

### 1.2 Paragangliomas

Paragangliomas are tumors arising from the paraganglia described above. They are frequently multifocal, especially in those patients with a hereditary component. Traditionally, they are divided in head-and-neck paragangliomas (also referred to as “glomus tumors”) and paragangliomas located in thorax and abdomen. However, in recent years new developments in genetics and diagnosis have made this distinction much less strict, as will be reviewed in this thesis.

In 2004 the World Health Organization (WHO) classification of endocrine tumors defined a pheochromocytoma as an intra-adrenal paraganglioma, whereas closely related tumors of extra-adrenal sympathetic or parasympathetic paraganglia are classified as extra-adrenal paragangliomas (4). For simplification, in the literature, the
term pheochromocytoma is often used to refer to both adrenal and sympathetic ganglia-derived extra-adrenal catecholamine secreting tumors. In general, about 80% of the sympathetic paragangliomas arise from the adrenal medulla (true pheochromocytomas) (5). Sympathetic extra-adrenal paragangliomas are most frequently found in the abdomen, pelvis and less often thorax and almost all produce and secrete catecholamines or their metabolites. On the other hand, head-and-neck paragangliomas, which were formerly referred to as glomus tumors, are derived from parasympathetic tissue and, therefore, rarely produce significant amounts of catecholamines.

Although the WHO nomenclature seems arbitrary, paragangliomas in different locations often display different clinical behavior, with a separate approach regarding diagnosis, treatment and follow-up needed (4), as will be further exemplified in this thesis.

1.3 Clinical presentation

1.3.1 Head-and-neck Paragangliomas

Head-and-neck paragangliomas are slow growing tumors, which are frequently reflected by a delayed diagnosis. An indolent neck mass is frequently the presenting symptom (6). The average age at presentation varies from 35 to 55 years (6-8). Carotid body tumors are located in the carotid bifurcation, whereas vagal body tumors can project into the lateral pharynx. Jugulo-tympanic paragangliomas are found in the jugular foramen or tympanic cavity and often present with hearing loss or pulsating tinnitus in an early stage compared to the other head-and-neck paragangliomas. Otoscopic investigation may reveal a reddish tumor behind the eardrum, that pulsates after application of pressure with a pneumatic otoscope, the so-called Brown’s sign (9). Furthermore, jugulo-tympanic tumors may present with invasion in the skull base (10). Although head-and-neck paragangliomas are usually benign tumors, their location in close proximity to nerves and vasculature in the head-and-neck region often results in considerable morbidity and cranial nerve dysfunction (6). In addition, patients with hereditary head-and-neck paragangliomas have an increased risk of developing paragangliomas at other locations like the adrenal medulla (pheochromocytoma) (11;12).

1.3.2 Pheochromocytomas and (sympathetic) extra-adrenal paragangliomas

The clinical presentation of pheochromocytoma and extra-adrenal paragangliomas is highly variable (13). Most symptoms are due to the elevated levels of catecholamines. Sustained hypertension is found in more than 60-90% of pediatric pheochromocytoma cases, whereas it is reported in 50% of adult cases. Pheochromocytoma is estimated to be prevalent in approximately 1% of hypertensive pediatric patients (14-18). In adults, the prevalence of pheochromocytoma or paraganglioma ranges from 0.1-0.6% in a patient population with hypertension (5). Palpitations, headache, excess sweating and
pallor are well-known effects of continuous or paroxysmal catecholamine excess (5;19). Signs and symptoms of sweating, nausea, vomiting, weight loss, polyuria, and visual disturbances have been reported more often in children than in adults (20;21). Anxiety may also be a presenting symptom, as exemplified in a recent brief report in a pediatric journal concerning two children with pheochromocytoma and behavioral symptoms similar to those seen with ADHD (22). Spells, if present, may vary widely from seconds to hours, as do their intervals and frequencies. The occurrence of attacks is unpredictable. Direct stimulation of the tumor (e.g. bladder localization), physical activity, diagnostic procedures, certain drugs or food may, however, trigger spells (5). Less frequent symptoms are constipation, flushing, fever and seizures. Furthermore, metabolic actions of catecholamines may lead to hyperglycemia and electrolyte disturbances as presenting symptoms, which could lead to delayed diagnosis or near-fatal complications (23;24). Although malignant pheochromocytomas and paragangliomas are considered rare, their prevalence is strongly dependent on the underlying mutation (25;26) (this thesis, chapter 5).

The high prevalence of hypertension in the normal (mostly adult) population and the variable nature of hypertension associated with pheochromocytomas, has led to several studies trying to identify which blood pressure characteristics should prompt further diagnostic imaging. Twenty four-hr blood pressure monitoring in pheochromocytoma subjects revealed higher blood pressure variability than in other hypertensive patients (27). Paroxysmal hypertension or the development of (orthostatic) hypotension against the background of sustained hypertension, or amelioration or even inversion of the circadian blood pressure rhythm (28) may be important clues for finding a pheochromocytoma. Case reports have reported epinephrine secreting pheochromocytomas presenting as a circulatory shock (29). Pathophysiologic mechanisms include intravascular volume depletion because of chronic high catecholamine levels, sudden tumor necrosis with consequent drop in catecholamine levels (5), desensitization of adrenoceptors (most likely due to long-term exposure to high circulating catecholamine levels), which was demonstrated in a rat model of pheochromocytoma (30) and the modulating effects of conjugation on catecholamines (31). This might be an explanation for the reported lack of correlation between the level of catecholamine excess and symptoms (32;33). Furthermore, tumors that secrete predominantly dopamine, often present with normotension (34).

1.4 Genetics

1.4.1 Head-and-neck Paragangliomas

Hereditary patterns in the occurrence of head-and-neck paragangliomas had since long been suspected, but it was van der Mey et al. who for the first time described a gender specific transmission of the disease, called maternal imprinting (35). Studying large Dutch families using linkage analysis led to the discovery of 2 loci: PGL1 on
chromosome 11q23 and PGL2 on chromosome 11q13 (36-38). Strong evidence for a common founder in head-and-neck paragangliomas in the Netherlands has been demonstrated (39). Eventually, the succinate dehydrogenase subunit D (SDHD) gene was identified as the cause of the hereditary head-and-neck paragangliomas with maternally imprinted inheritance (40). Succinate dehydrogenase subunit D is one of the four subunits (subunit A, B, C and D) of mitochondrial complex II, which is involved in two mitochondrial pathways: the inner mitochondrial membrane bound electron transport chain and the mitochondrial matrix associated Krebs tri-carboxylic-acid (TCA) cycle (Figure 2). The interaction between both is necessary for maximal efficiency in ATP (energy) production under aerobic conditions (41). In following years, mutations in the subunits B (chromosome 1p36) and C (chromosome 1q21) have been found to be associated with paraganglioma syndromes as well (11;42). These mutations have an autosomal dominant pattern of inheritance. Patients with subunit C mutations have predominantly head-and-neck paragangliomas without paragangliomas at other locations (43).

**Figure 2: Inner mitochondrial membrane complexes and the electron transport chain**

In addition to their role in energy metabolism, mitochondria are also thought to play a role in regulating apoptosis. Mutations in the SDH genes may lead to partial assembly or disassembly of complex II and result in alteration in membrane composition with a
subsequent change in resistance to apoptosis (41). In addition, reduced SDH activity leads to increased levels of reactive oxygen species (ROS), which have been reported to activate the hypoxia-inducible factors (HIF-1α), which could lead to reduced apoptosis and increased cell proliferation via several mechanisms (44-46). Because of the founder effect and a long history of research in head-and-neck paragangliomas in Leiden, a large cohort of patients associated with the SDHD mutation was collected in Leiden, which is the focus of this thesis.

1.4.2 Pheochromocytomas and (sympathetic) extra-adrenal paragangliomas

According to recent reports, at least 25% of sympathetic paragangliomas have a hereditary basis (47). In children, this percentage is even higher and is estimated to be approximately 40%. Therefore, genetic testing should be performed in every child with paragangliomas, and should strongly be considered in adults. An overview of the main clinical characteristics of previously described hereditary syndromes is provided in Table 1 (5).

The location of tumors, presence of metastases and the biochemical profile of catecholamine secretion may contribute to the a-priori chances of finding a specific mutation due to specific genotype-phenotype interactions. Of course, specialized genetic consultation to collect family history, outline repercussions of genetic testing and obtain informed consent is mandatory (4). Family screening should be offered when appropriate.

**Succinate dehydrogenase**

As was described above, mutations in the genes encoding subunits of mitochondrial complex II succinate dehydrogenase are associated with familial head-and-neck paragangliomas. In recent years, these mutations have been found to be associated with pheochromocytomas and extra-adrenal paragangliomas as well. In the literature, in approximately 3-11% of patients with a ‘sporadic’ pheochromocytoma one of these subunit mutations can be found. Carriers of the SDHD mutation often present with head-and-neck paragangliomas and multifocal disease and have a lifelong estimated risk to develop head-and-neck paragangliomas of ~100% (25). In recent years, van Houtum et al. reported an estimated risk of 20% for developing predominantly norepinephrine secreting paragangliomas at other locations in the Leiden head-and-neck paraganglioma cohort (12). The chances for SDHD associated malignant disease have not been fully elucidated, so far. The SDHB mutation not only predisposes patients to develop head-and-neck paragangliomas (lifetime risk ~35%)(25), but also to have extra-adrenal localizations (lifetime ~75%)(25) and metastatic disease (up to 30-50%) (11;25;26;48). The predominant biochemical phenotype is hypersecretion of norepinephrine and/or dopamine (26).
**Table 1: Clinical characteristics of hereditary syndromes associated with pheochromocytomas or extra-adrenal paragangliomas**

<table>
<thead>
<tr>
<th><strong>Paraganglioma syndromes (SDH)</strong></th>
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<tr>
<td>SDHB/C/D</td>
<td>Head-and-neck paragangliomas (especially in SDHC, SDHD)</td>
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<td></td>
<td>Intra-adrenal paragangliomas (pheochromocytomas)(SDHB, SDHD)</td>
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<tr>
<td></td>
<td>Extra-adrenal paragangliomas (SDHB, SDHD)</td>
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<td></td>
<td>Malignancy rate (mainly SDHB, up to 70%)</td>
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<thead>
<tr>
<th><strong>Multiple Endocrine Neoplasia type 2</strong></th>
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<tr>
<td><strong>Type 2a.</strong></td>
<td>Medullary thyroid carcinoma</td>
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<tr>
<td></td>
<td>Pheochromocytoma</td>
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<tr>
<td></td>
<td>Hyperparathyroidism</td>
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<tr>
<td></td>
<td>Cutaneous lichen amyloidosis</td>
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<tr>
<td><strong>Type 2b.</strong></td>
<td>Medullary thyroid carcinoma</td>
</tr>
<tr>
<td></td>
<td>Pheochromocytoma</td>
</tr>
<tr>
<td></td>
<td>Multiple neuromas</td>
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<td></td>
<td>Marfanoid habitus</td>
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<tr>
<th><strong>Von Hippel-Lindau syndrome type 2</strong></th>
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<tr>
<td><strong>Type 2a.</strong></td>
<td>Retinal and central nervous system haemangioblastomas</td>
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<td></td>
<td>Pheochromocytoma</td>
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<tr>
<td></td>
<td>Endolymphatic sac tumors</td>
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<tr>
<td></td>
<td>Epididymal cystadenomas</td>
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<tr>
<td><strong>Type 2b.</strong></td>
<td>Renal-cell cysts and carcinomas</td>
</tr>
<tr>
<td></td>
<td>Retinal and central nervous system haemangioblastomas</td>
</tr>
<tr>
<td></td>
<td>Pancreatic neoplasms and cysts</td>
</tr>
<tr>
<td></td>
<td>Pheochromocytoma</td>
</tr>
<tr>
<td></td>
<td>Endolymphatic sac tumors</td>
</tr>
<tr>
<td></td>
<td>Epididymal cystadenomas</td>
</tr>
<tr>
<td><strong>Type 2c.</strong></td>
<td>Pheochromocytoma</td>
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<tr>
<th><strong>Neurofibromatosis type 1</strong></th>
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<tr>
<td>Neurofibromas (multiple)</td>
<td></td>
</tr>
<tr>
<td>Café-au-lait spots</td>
<td></td>
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<tr>
<td>Pheochromocytoma</td>
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Adapted from: Lenders *et al.* Lancet 2005
Multiple Endocrine Neoplasia type 2

The tumor syndrome multiple endocrine neoplasia type 2 (MEN-2) is the result of activating mutations of the RET proto-oncogene and is inherited in an autosomal dominant fashion. The gene is located on chromosome 10q11.2 and encodes a receptor tyrosine kinase. Pheochromocytomas associated with this syndrome often produce both epinephrine and norepinephrine (49). Bilateral pheochromocytomas will develop in 50-80% of patients, but malignant pheochromocytoma is rare. Hypertension, if present, is more often of the paroxysmal type.

Von Hippel-Lindau

In von Hippel-Lindau disease (VHL) the mutation is located on chromosome 3p25-26 and inherited in an autosomal dominant fashion. In tumors with VHL mutations, the mutated VHL gene product stabilizes hypoxia-inducible factor HIF-1α under normoxic conditions (50;51). Remarkably, as was described above, HIF-1α appears to be upregulated in patients with SDH mutations, thus revealing a possible link in pathogenesis (45). Pheochromocytomas are present in 10-20% of patients with this syndrome (52). Although the reported mean age of presentation is reported to be 30 years, multiple cases of pediatric pheochromocytoma patients with VHL have been described (20;53-55). These pheochromocytomas are often (but not exclusively) located bilaterally in the adrenals and predominantly produce norepinephrine. Malignant disease is rare.

Neurofibromatosis type 1

This characteristic clinical syndrome is caused by a mutation in a gene on chromosome 17q11.2. The inheritance is autosomal dominant. Pheochromocytomas are rare (<5%) and usually produce a combination of epinephrine and norepinephrine. Because the clinical syndrome is so characteristic, genetic testing for the NF1 mutation should not be performed routinely.

1.5 Biochemical Diagnosis

The first step in patients with suspected pheochromocytoma and extra-adrenal paragangliomas is biochemical testing. Biochemical testing should be performed not only in symptomatic patients and patients with an adrenal incidentaloma, but also as a means of screening in subjects who have a hereditary risk for developing paragangliomas or recurrent disease. Traditional biochemical tests consist of measurement of 24-hour urinary and plasma catecholamines (norepinephrine, epinephrine and dopamine), and the degradation product urinary vanillylmandelic acid (VMA). However, the catecholamine metabolites normetanephrine and metanephrine (derived from norepinephrine and epinephrine, respectively) are produced continuously and independently of catecholamine release by intracellular O-
methylation after leakage of the parent amines from chromaffin granule stores in the cytoplasm (Figure 3) (56). Therefore, these metabolites are more accurate tests to diagnose pheochromocytoma than the conventional catecholamines (4,57). Quantification of plasma free metanephrine and normetanephrine or measurement of 24-hour urinary fractionated metanephrines are currently considered to be the most accurate biochemical tests for pheochromocytoma (Table 2) (58-60). Reference intervals for metanephrines should primarily ensure optimum diagnostic sensitivity, with specificity as a secondary consideration to avoid the deadly consequence of a missed diagnosis (4). Furthermore, biochemical diagnosis of pheochromocytoma in children requires age-appropriate reference intervals (59). Especially the rate of urinary excretion of catecholamines and metanephrines in young children can be less than a third of those in adults. In some cases measurement of dopamine or its metabolite methoxytyramine may have diagnostic use and indicate malignant tumor potential (61).

Table 2: Sensitivity and specificity of biochemical tests

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Hereditary</td>
<td>Sporadic</td>
</tr>
<tr>
<td>Plasma metanephrines</td>
<td>97</td>
<td>99</td>
</tr>
<tr>
<td>Plasma catecholamines</td>
<td>69</td>
<td>92</td>
</tr>
<tr>
<td>Urinary catecholamines</td>
<td>79</td>
<td>91</td>
</tr>
<tr>
<td>Urinary fractionated metanephrines</td>
<td>96</td>
<td>97</td>
</tr>
<tr>
<td>Urinary total metanephrines</td>
<td>60</td>
<td>88</td>
</tr>
<tr>
<td>Urinary vanillylmandelic acid</td>
<td>46</td>
<td>77</td>
</tr>
</tbody>
</table>

Adapted from: Lenders et al. 2002 (reference 57) and Zelinka et al. 2007 (reference 58).

However, to preserve high diagnostic sensitivity it is strongly recommended to obtain blood samples in the supine position (62). Tricyclic antidepressants, beta-blockers, calcium-antagonists and acetaminophen may either influence catecholamine levels or interfere with biochemical testing (5). To distinguish true positive from false positive
results, it is reasonable to take into account the level of elevation. On a Consensus meeting it was stated that testing algorithms should not simply rely on a binary approach for test interpretation (i.e. a result being positive or negative), but should take advantage of the continuous nature of biochemical test results (4). In selected cases with persistent minor increases in plasma free normetanephrine, clonidine suppression testing might be needed for accurate interpretation of elevated catecholamine levels (13). Clonidine given orally suppresses release of norepinephrine by activating the $\alpha_2$-adrenoceptors in the brain and on sympathetic nerve endings (63). Because of possible severe hypotension, and complex interpretation of the results, this test should only be performed by experienced clinicians. Suppression of plasma normetanephrine by 40% (norepinephrine by at least 50%) or more or to below the upper reference limit virtually excludes the diagnosis of pheochromocytoma (64).

1.6 Tumor localization studies

In patients presenting with an indolent neck mass or complaints and physical examination suggestive of head-and-neck paragangliomas, a head-and-neck MRI is performed. Because of superior sensitivity head-and-neck MRI is to be preferred over ultrasound or CT scan (65;66).

Localization studies to demonstrate pheochromocytomas should be performed after a conclusive biochemical diagnosis has been made. However, in patients with a hereditary predisposition for pheochromocytoma, even lower or normal levels of catecholamines may warrant further diagnostic imaging as a means of screening because of a high a-priori chance for developing (non-secreting) paraganglioma or malignant disease (4). Since most tumors arise in the abdomen, either in- or outside the adrenals, we recommend starting with a CT/MRI abdomen and pelvis. Although MRI has excellent sensitivity (90-100%) the ability of MRI to specify a pheochromocytoma from other abdominal lesions can be insufficient (67). Therefore, additional functional imaging using $^{123}$I-MIBG scintigraphy should be performed to confirm the diagnosis and to look for paragangliomas in multiple locations or clues for malignant disease (68). $^{123}$I-MIBG scintigraphy offers an excellent specificity (95-100%) and reasonable sensitivity for detecting pheochromocytomas (69;70). However, because of the high prevalence of genetic mutations associated with multifocal disease, others believe $^{123}$I-MIBG scintigraphy should be performed unless contra-indicated otherwise (4;53). Medication use should be checked before $^{123}$I-MIBG imaging, because concurrent use of medications like labetalol or tricyclic antidepressants (TCA) is able to interfere with uptake of the tracer by the tumor (71). Furthermore, it has been reported that malignant paragangliomas loose the ability to accumulate MIBG, thus reducing the value of this isotope in metastatic disease. In these patients other means of imaging like $^{111}$In-Octreotide scintigraphy, $^{18}$F-fluorodopamine or $^{18}$F-fluorodeoxyglucose positron emission tomography (PET) may be of additional value (70;72).
Figure 3: Pathways of metabolism of catecholamines to free and sulfate-conjugated metanephrines

![Diagram of metabolic pathways]

Courtesy of: Dr. G. Eisenhofer. PNMT, phenylethanolamine N-methyltransferase; COMT, catechol-O-methyltransferase; SULT1A3, monoamine-preferring sulfotransferase.

Recently, Timmers et al. reported FDG-PET to be a superior tool in the evaluation of metastatic SDHB-associated adult pheochromocytomas and paragangliomas (73). However, in most cases the combination of MRI and [123I]-MIBG will be sufficient for diagnosis. The current standing of MIBG among the newer PET imaging agents in pheochromocytoma and paraganglioma diagnosis will be reviewed in chapter 7 of this thesis.

1.7 Treatment

1.7.1 Treatment of head-and-neck paragangliomas

Because of their location close to vasculature and nerves, damage can be inflicted both by the tumor growth itself and surgery. Surgical complications however, tend to be limited in tympanic tumors and carotid body tumors confined to the bifurcation (6). Radiotherapy and embolization have been used to treat head-and-neck paragangliomas as well, but are out of the scope of this thesis. According to Jansen et al. (6) it is justified to focus on prevention of morbidity rather than to focus on tumor removal itself. In this...
thesis we therefore specifically address the quality of life in patients with head-and-neck paragangliomas (chapters 2 and 3). However, the risk of developing pheochromocytomas and extra-adrenal paragangliomas must be taken into account in every one of these patients (chapter 4).

1.7.2 Treatment of pheochromocytomas and (sympathetic) extra-adrenal paragangliomas

Surgery is the mainstay of pheochromocytoma and extra-adrenal paraganglioma treatment, but intra-operative risks must be kept to a minimum by appropriate pre-operative medical treatment to block the effects of catecholamines for at least 10-14 days before surgery (4). In a study by Goldstein et al. adequate pre-operative α-blockade reduced the number of peri-operative complications from 69% to 3% (74). Several different protocols have been proposed with α-blockade using either doxazosine or phenoxybenzamine (33). Use of the non-competitive α-blocker, phenoxybenzamine, has been reported to have advantages over using competitive blockers like doxazosine, which could theoretically be displaced by excessive catecholamine releases during surgery. However, post-operative hypotension as a side-effect occurs more frequently with phenoxybenzamine because of a longer half-life. In our view, calcium channel blockers could be useful in situations where the use of α-blockade alone is not sufficient to control blood pressure or the extent of side effects outweighs the benefit of α-blockade. It is very important to emphasize that, because of the risk for unopposed α-receptor stimulation, β-blockers for residual tachycardia may only be started after adequate pre-treatment with α-blockade (75). The catecholamine synthesis inhibitor α-methyl paratyrosine (methyrosine) should be used with caution. Possible side effects are diarrhea and crystalluria especially with higher dosages. Because it crosses the blood-brain barrier, sedation and extra-pyramidal symptoms (rare) may occur (19). Different criteria have been proposed to ensure adequate preoperative preparation. At the National Institutes of Health, Bethesda, United States, the goal is to achieve preoperative blood pressure of 130/80 mm Hg or less while sitting and about 100 mm Hg systolic while standing (not less than 80/45 mm Hg) and target heart rate of about 60–70 beats per minute (bpm) while sitting and 70–80 bpm while standing (75). Increasing the phenoxybenzamine dosage until there is an orthostatic drop (76) or ‘normalization’ of blood pressure has been advocated. In Leiden we have a similar goal, but patients are admitted five days before surgery and doxazosine instead of phenoxybenzamine is administered in increasing dosages until an orthostatic drop develops. Patients with pheochromocytoma experience volume contraction from chronic vasoconstriction. Therefore, pre-operative volume expansion achieved by saline infusion or increased water intake is recommended to reduce post-operative hypotension (21). Furthermore, pre-operative correction of possible potassium abnormalities due to high renin levels might be necessary. Glucose levels should especially be monitored closely in the peri-operative setting, because of the risk for
postoperative hypoglycemia (21;75). Laparoscopic adrenal resection by an experienced surgeon is the preferred treatment (21;77) and laparoscopic cortical sparing adrenalectomies must be considered in patients with bilateral disease (or a genetic risk to develop bilateral tumors) to avoid chronic glucocorticoid deficiency (78). However, open surgical approaches will sometimes be necessary in patients with large tumors (>6 cm), tumors that are difficult to reach laparoscopically and extensive locally invasive or metastatic disease.

### 1.8 Malignant disease

Because there is no definite histological substrate for malignant pheochromocytoma and paraganglioma, malignant disease can only be established by either demonstrating local tumor invasion and/or the presence of paraganglioma cells outside the normal sites. Most frequent sites of distant metastases are bones, lung and liver (79;80). Because metastatic pheochromocytomas and paragangliomas can not be cured, treatment should be considered when quality of life is influenced by catecholamine excess or the metastatic lesion itself. Tumor debulking, embolization, systemic therapy with $[^{31}\text{I}]$-MIBG or chemotherapy (mostly a combination of cyclophosphamide, vincristine, dacarbazine) can provide tumor regression and symptom relief in patients (79), which is usually short-lived (5-yr-survival ~50%), although long-term remissions have been described (81;82). Clinicians using these therapies should be aware of the risk of bone marrow depression and potentially fatal complications due to a sudden increase in catecholamine levels secondary to tumor necrosis. The use of $[^{177}\text{Lu-DOTA}]-\text{Octreotide}$ in malignant paraganglioma has only been described in case reports (83).

### 1.9 Scope of this thesis

In recent years, improvements in diagnosis, genetics, localization, and treatment of paragangliomas have changed dramatically the approaches to these tumors. In the recent past, the SDHD mutation was thought to be only associated with hereditary head-and-neck paragangliomas. These paragangliomas were often regarded as a rare benign disease of the head-and-neck with little need for aggressive follow-up. The Dutch founder effect and the historical interest in research of these tumors in the Leiden area, has resulted in a unique, large cohort of head-and-neck paraganglioma patients in Leiden. In total, 7 theses regarding head-and-neck paragangliomas have already been approved at the Leiden University. Recently, the prevalence of potentially life-threatening catecholamine secreting paragangliomas at locations outside the head-and-neck area was found to be much higher than previously expected. This proved the value of a more multidisciplinary approach that was instituted in the Leiden University Medical Center. This thesis is the first to specifically address the endocrine aspects of familial paraganglioma/pheochromocytoma syndromes.
In this thesis, the following aspects of the clinical paraganglioma / pheochromocytoma syndromes will be addressed:

- Quality of life and sleep
- Outcome of screening for pheochromocytoma in SDHD-associated head-and-neck paragangliomas; clinical characteristics, biochemical phenotype and diagnostic imaging
- Malignant dedifferentiation in SDHD-associated disease
- Mediastinal paragangliomas in SDHx-associated disease
- Current standing of MIBG scintigraphy and future role of PET imaging

**Quality of life and disturbed sleep**

The effects of disease and its treatment can be assessed with health related Quality of Life studies. Although patients with head-and-neck paragangliomas most often have a benign disease, patients often report several complaints. In his thesis J.C. Jansen concluded that the approach to head-and-neck paragangliomas must be focused on prevention of morbidity rather than tumor removal (6). The effect of paragangliomas on Quality of Life, however, had not been previously investigated in head-and-neck paraganglioma patients. Therefore, in chapter 2 we compared quality of life assessments using validated questionnaires between patients with head-and-neck paragangliomas and control subjects. Because fatigue was one of the reported complaints in that study and carotid bodies are known to be involved in oxygen sensing, which is involved in the regulation of sleep, we hypothesized that sleep quality might be disturbed in these patients. Therefore, we compared subjective sleep characteristics between patients with head-and-neck paragangliomas and control subjects using validated sleep questionnaires in chapter 3.

**Outcome of screening for pheochromocytoma**

In 2005 we reported that the prevalence of catecholamine excess in our SDHD-linked HNP patients was much higher than previously appreciated (12). Therefore, all our patients are offered structured follow-up at regular intervals with screening for catecholamine excess with subsequent imaging and therapy if necessary. Although some studies have investigated genotype-phenotype correlations in SDHD mutation carriers (25;48), these were multi-center referral based patients with diverse underlying SDHD mutations. This may have induced referral bias in the interpretation of the results. We have one of the largest, single-center cohorts with SDHD-linked head-and-neck paragangliomas in the world, which enabled us to specifically assess the outcome of screening in these patients. In chapter 4 we evaluated the clinical
characteristics, biochemical phenotypes and imaging results in this large cohort of patients and report a very high prevalence of pheochromocytomas and extra-adrenal paragangliomas in SDHD associated patients.

Malignant paragangliomas associated with SDHD

In previous genotype-phenotype studies malignant paragangliomas were frequently found to be associated with SDHB mutations. On the other hand SDHD related paragangliomas were rarely reported to be malignant (25;48). Therefore, we evaluated in chapter 5 the clinical characteristics of malignant paragangliomas associated with SDHD mutations.

Mediastinal paragangliomas associated with SDHx mutations

Approximately 20 percent of the catecholamine-producing tumors is derived from extra-adrenal chromaffin tissues, which are termed extra-adrenal paragangliomas. Although paragangliomas are found mostly in the abdomen, they are less commonly found in the pelvic sympathetic plexus of the urinary bladder, and only in ~ 2 percent of the cases in the mediastinum. In chapter 6 we present data of mediastinal paragangliomas and found them to be frequently functional, metabolically active, and associated with SDHx mutations. We describe detailed data on clinical characteristics and biochemical phenotype.

Current standing of MIBG scintigraphy and future role of PET imaging

With the identification of several different clinical phenotypes, MIBG scintigraphy was found to have a different performance in subsets of pheochromocytoma / paraganglioma patients. Reduced sensitivity of MIBG scintigraphy in some familial paraganglioma syndromes, malignant disease and extra-adrenal paragangliomas has been found. Newer compounds for PET imaging have emerged and were found to be superior in certain cases. In chapter 7 we present a concise review of the historic role of MIBG and its future prospects among the newer maging methods in paragangliomas.
Chapter 1

References

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Chapter 1

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