SDHB-linked Paraganglioma

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SDHB-linked Paraganglioma

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SDHB-linked Paraganglioma

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SDHB–linked PARAGANGLIOMA

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door

Johannes Adriaan Rijken

geboren te Veenendaal

and as ye would that men should do to you,
do ye also to them likewise.

Luke 6:31 King James Version

voor mijn ouders

Promotiecommissie

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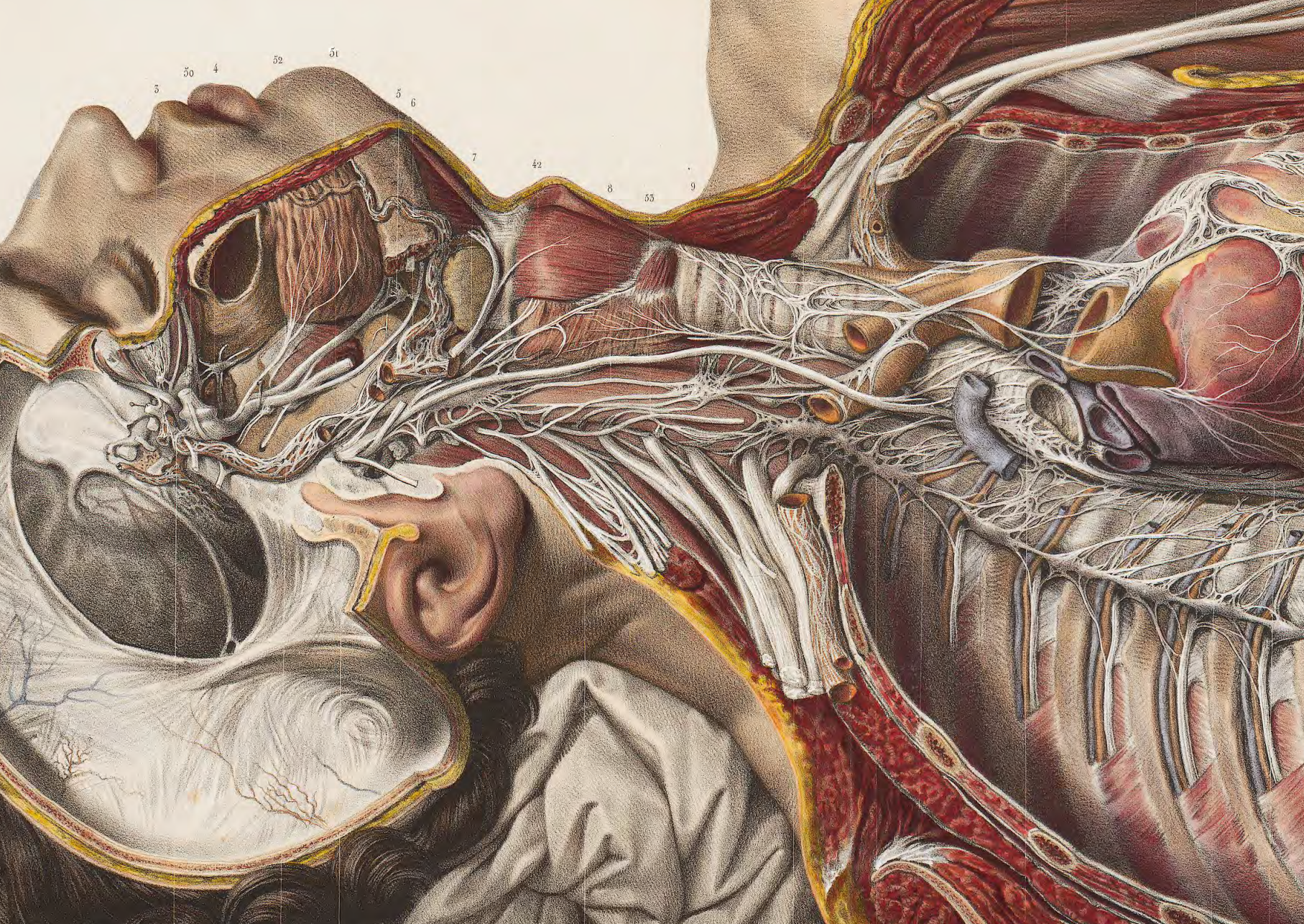
CONTENTS


3
50
4
52
51
5
6
7
42
8
53
9

1 General introduction

1.1 THE PARAGANGLION SYSTEM

Paraganglia are anatomically widely distributed cell clusters of neuroectodermal origin that are associated with the autonomous nervous system. The paraganglion system consists of the adrenal medulla, the largest paraganglion in the human body, the sympathetic paraganglia, and the parasympathetic paraganglia[1]. The sympathetic paraganglia are associated with the ganglia of the paravertebral sympathetic trunk, the organ of Zuckerkandl, and the celiac, renal, suprarenal and hypogastric plexuses (figure 1.1 left). The parasympathetic paraganglia consist of the intravagal bodies and the branchiomeric paraganglia in the mediastinum and head and neck region, most notably located in the carotid bifurcation, the jugular foramen and on the promontory of the middle ear (figure 1.1 right).

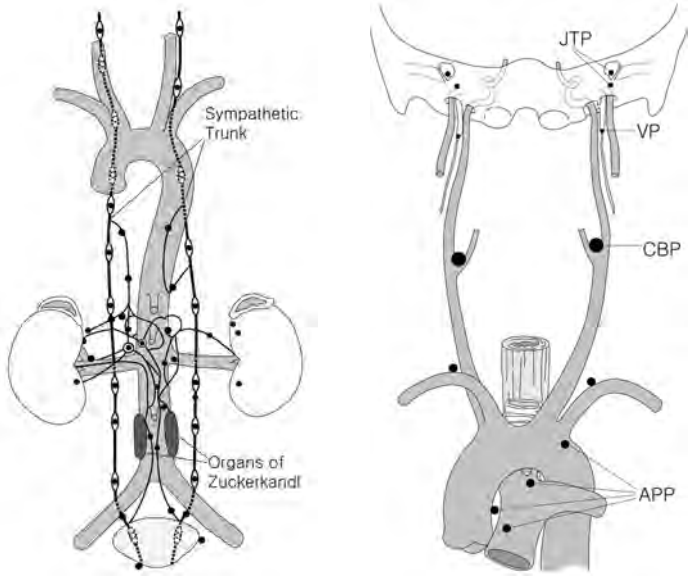


Figure 1.1 The paraganglion system.

Drawings show the anatomic distribution of healthy extra-adrenal paraganglia connected with the sympathetic system (left) and parasympathetic system (right). APP = aorticopulmonary paraganglia, CBP = carotid body paraganglion, JTP = jugulotympanic paraganglia (located in the jugular foramen and on the promontory of the middle ear), VP = vagal paraganglia. Adapted from: Lee *et al.* Am. J. Roentgenol. 2006;187:492-504.

The exact function of the paraganglion system is not fully known. The adrenal medulla, the inner part of the adrenal gland, produces the catecholamines epinephrine, norepinephrine, and dopamine. These hormones regulate heart rate, blood pressure, metabolism, and cause vasoconstriction and bronchial dilatation. The organs of Zuckerkandl are thought to be important regulators of the embryonic homeostasis and blood pressure through the production and release of catecholamines during early gestation, and they normally start to regress in the third trimester[2].

Kohn recognized the similarity between sympathetic paraganglia and the carotid body[3]. The carotid body is the best-studied head and neck paraganglion, which is visible macroscopically as a flattened rice grain-shaped organ. This paraganglion is situated medially in the adventitial plane of the carotid bifurcation and a fibrovascular pedicle (Mayer's ligament) may be seen carrying the small glomic arteries and myelinated nerve bundles. Microscopically, the carotid body is composed of multiple ovoid lobules separated by fibrous septa that contain abundant myelinated nerve fibers and small arteries that supply the individual lobules. Each lobule is organized in several nests of parenchymal chief cells (type I cells) interspersed with stroma that contains nerve endings, small arterioles and venules. At the periphery of the cell nests a second cell type, the sustentacular cell (type II cell), is present that is believed to have supportive function. Type II cells are extremely rare in paraganglia, other than at the carotid bifurcation[4]. The typical nested architecture of chief cells and sustentacular cells, surrounded by a highly vascular stroma, is a prominent feature of branchiomeric paraganglia and is termed 'Zellballen' (figure 1.2)[5].

The ability of paraganglia to synthesize, store and secrete catecholamines (epinephrine, norepinephrine, and dopamine) is reflected by a positive chromaffin reaction of chromates with these compounds if present in sufficient quantity. The reaction can be seen with a light microscope and paraganglionic tissue is often said to be *chromaffin*, which is not always the case[6].

The carotid and aortic bodies function as peripheral chemoreceptors sensitive to changes in arterial oxygen levels and, to a lesser degree also to carbon dioxide levels and arterial pH. Arterial hypoxia, hypercapnia and acidosis cause excitation of the paraganglionic type I cells. This signal is relayed by the afferent fibers of the glossopharyngeal and vagal nerves to the central cardiorespiratory centers in the medulla oblongata, which regulate cardiac output and respiration[7].

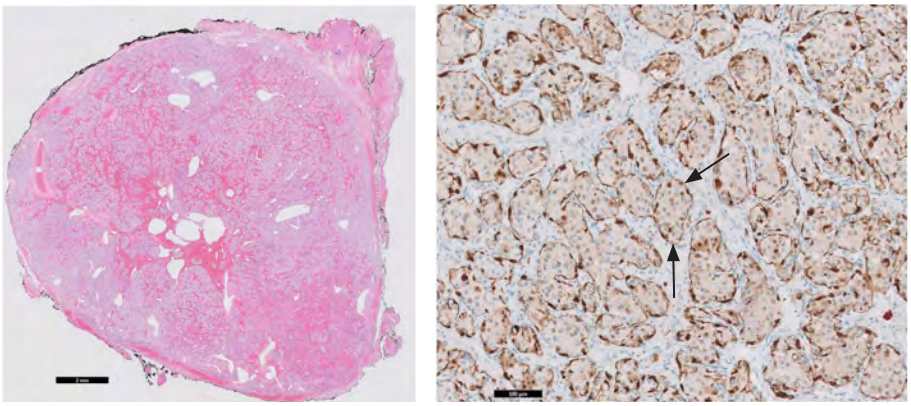


Figure 1.2 Microscopy of paraganglioma tissue showing the type I and type II cells in the classic Zellballen configuration. This characteristic architecture is usually preserved in the progression from normal paraganglion tissue to paraganglioma.
Left = overview of hematoxylin and eosin (H-E) stained section of paraganglioma tissue. Right = immunohistochemical staining with positivity for S-100 protein shows the typical nested architecture of chief cells and sustentacular cells, surrounded by a highly vascular stroma, termed 'Zellballen' (indicated by arrows).

1.2 PARAGANGLIOMAS

Neoplastic transformation of paraganglia results in the development of paragangliomas (PGLs). PGLs are hypervascular tumors that can arise in the various locations of the paraganglion system. They are usually benign, slow growing, and the majority (circa 90%) of tumors occur in the adrenal paraganglia, so-called pheochromocytomas (PCCs). PGLs are divided into two groups: one originating from the parasympathetic system and one from the sympathetic system. Parasympathetic PGL are primarily located in the head and neck region and less frequently in the thorax, abdomen and/or pelvis. PCCs and sympathetic PGLs (sPGLs) are tumors arising from neural crest tissue that develops into paraganglia throughout the body. Approximately 85% of sPGL occur in the abdomen, 12% in the thorax, and 3% in the head and neck[8].

Head and neck paragangliomas

Epidemiology

Head and neck paragangliomas (HNPGLs) are rare neoplasms. Estimates of the clinical incidence vary between 1/1.000.000 and 1/100.000[9-11]. These figures may represent an underestimation because of the often asymptomatic and clinically favorable nature of PGLs. Necroscopy rates for carotid body PGLs of 1:13.400

to 1:3.860 point towards a higher incidence, but may represent an overestimation of the true incidence in the general population[10,12]. Several studies have reported a female predominance, especially in series of carotid body tumors and among high altitude dwellers, possibly due to differences in the development of chemoreceptive-reflexes between males and females[11,13-15].

Localization

HNPGLs most frequently originate from the paraganglia in the bifurcation of the carotid artery, the jugular foramen, along the vagus nerve or along the tympanic nerve (figure 1.3)[16].

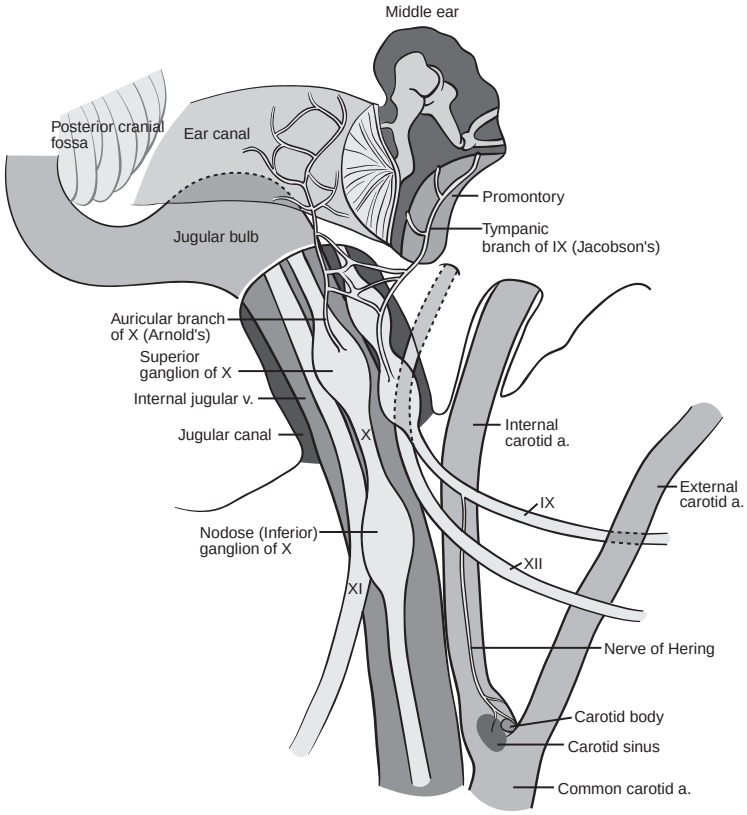


Figure 1.3 Schematic representation of common sites of head and neck paragangliomas and their relationship to the lower cranial nerves and major vessels. IX = glossopharyngeal nerve, X = vagus nerve, XI = spinal accessory nerve and XII = hypoglossal nerve. The branch of glossopharyngeal nerve to the carotid sinus (nerve of Hering) is a small nerve in the neck, which innervates the carotid sinus and the carotid body. From Persky MS, Hu KS. Paragangliomas of the head and neck. In: Harrison LB, Sessions RB, Hong WK, eds. Head and Neck Cancer: A Multidisciplinary Approach. 3rd ed. Lippincott Williams and Wilkins, Philadelphia, PA; 2009. Reprinted with permission by Wolters Kluwer.

HNPGLs are named according to the anatomical site of origin. PGLs originating at the site of the carotid body between the internal and external carotid artery are termed *carotid paraganglioma* or *carotid body tumor*. PGL associated with the vagus nerve is referred to *vagal paraganglioma*. PGLs of the jugular bulb, involving the temporal bone are named *jugular paraganglioma* or *jugulotympanic paraganglioma*. These tumors develop around the jugular bulb cranial to the parapharyngeal space, usually involving the temporal bone. They may extend along the great vessels into the parapharyngeal space. Very large jugular PGLs may be difficult to distinguish form vagal PGLs because vagal PGLs most commonly arise high in the neck adjacent to the vagal ganglion[17]. *Tympanic paragangliomas* arise in the middle ear along the course of Jacobson's or Arnold's nerve. These lesions can vary from small masses on the cochlear promontory to tumors that extend into the mastoid and external auditory canal. Carotid body PGLs are the most common PGLs encountered in the head and neck area, and accounts for over half of the HNPGLs. PGLs in the larynx, nasal cavity, orbit, trachea, aortic body, lung, and mediastinum have also been described[18].

Signs and symptoms

HNPGLs generally present in mid-adult life as asymptomatic space occupying lesions. These tumors can become symptomatic and symptoms vary with tumor size and localization. Generally they exhibit a slow rate of growth with the potential to remain stable and thus in the majority of cases clinically silent over years. Reports have suggested that tumors, which have been followed radiographically, show an increase in size of less than 5 millimeter per year[19]. Approximately 10-15% show a more aggressive behavior with rapid progression[20]. Overall the most common symptom is a painless, palpable, lateral neck mass or pharyngeal bulging. With further progression, a HNPGL may compress or involve the cranial nerves, especially of the facial (VIIth), glossopharyngeal (IXth), vagal (Xth), spinal accessory (XIth) and hypoglossal (XIIth) nerves, because of their close relationship with the jugulotympanic, vagal and carotid paraganglia (figure 1.3). Subsequently speech and swallowing deficits (hoarseness and dysphagia) and sometimes aspiration may occur[21]. A conductive hearing loss and tinnitus (typically pulsatile) may be present in case of jugulotympanic or tympanic PGL. HNPGLs are of parasympathetic origin and the majority is 'non-functional', i.e. does not secrete catecholamines. However, up to 30 percent of HNPGLs does hypersecrete catecholamines, which may cause symptoms such as hypertension, paroxysmal palpitations, headache, agitation, excess sweating and/or pallor and in rare cases stroke, myocardial infarction or even death (see subheading '1.2.7 Management of functional head and neck paragangliomas')[22-25].

Diagnosis

The evaluation for HNPGL starts with a careful history, including family history of neck masses or surgery for head and neck tumors. A thorough examination of the ears, oral cavity, pharynx, larynx, neck and cranial nerve function is performed. Imaging is of paramount importance in patients with a clinical suspicion of HN-PGLs and/or in carriers of a pathogenic gene variant associated with the development of PGL (see also '1.3 Genetics of paragangliomas').

Ultrasound is typically utilized early in the diagnostic process of a palpable neck mass. Sonographic evaluation in case of HNPGL demonstrates a well-defined, heterogeneously hypoechoic mass, with marked internal vascularity on color Doppler imaging. Ultrasound can be helpful in performing a fine needle aspiration cytology that might be useful in the differential diagnosis, especially between PGL and squamous cell carcinoma or lymphoma (see below).

Magnetic resonance imaging (MRI) is the most important imaging technique for characterization and evaluation of HNPGL because of its good visualization of soft tissues. HNPGLs typically demonstrate hypointense signal on T1-weighted sequences and isointense to hyperintense signal on T2-weighted sequences. Internal flow voids are commonly seen, particularly on T2-weighted sequences. More rarely, areas of hyperintense intratumoral hemorrhage can be seen on both T1- and T2-weighted sequences. Hypointense flow voids and hyperintense areas of hemorrhage may result in a characteristic 'salt and pepper' appearance which may be apparent in tumors greater than 1centimeter. HNPGLs usually demonstrate avid, homogenous enhancement after administration of intravenous gadolinium contrast agents. The most accurate MRI technique in the detection of HNPGL is a pre- and post-contrast enhanced 3D Time of Flight (TOF) MR angiography[26,27].

On computed tomography (CT) HNPGLs present as a well-defined soft tissue attenuation masses. Commonly, these tumors demonstrate homogenous, avid enhancement after administration of intravenous contrast, though heterogeneity can occur in lesions with intratumoral thrombosis or hemorrhage. CT is superior to MRI for assessment of osseous involvement and evaluation of bony erosion at the skull base including the temporal bone and jugular fossa, particularly in the case of jugulotympanic PGL. The disadvantages of CT imaging for patients are the exposition to radiation and the use of contrast, which might provoke catecholamine release in patients that are not pre-treated with alpha- or beta-blockers[28]. Angiography, either with CT angiography (CTA), MR angiography (MRA), or digital subtraction angiography (DSA) is typically performed either as an adjunct to CT or MRI, as well as in the preoperative setting[29]. These modalities allow for evaluation of tumor

perfusion and identification of feeding vessels (for HNPGL usually arising from the ascending pharyngeal artery), which can guide subsequent embolization or surgical approaches[30]. There has been controversy concerning the usefulness of preoperative embolization. Some authors prefer routine preoperative embolization because it can lower blood flow and decrease tumor size, particularly in larger tumors. Others disagree on preoperative routine embolization due to post-embolization morbidity such the potential risk of stroke by embolic particles[31]. Angiography may also be used to perform a preoperative balloon occlusion test of the internal carotid artery. This test predicts tolerance for permanent occlusion of the internal carotid artery, in case preservation is not possible during surgery.

Nuclear medicine imaging techniques can be used to evaluate multicentric or metastatic PGL disease, including ^{131}I- and ^{123}I-metaiodobenzylguanidine (MIBG), ^{111}In-octreotide, and ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET), demonstrating focally increased uptake within the lesions[32-35]. ^{123}I-MIBG scintigraphy, despite its high specificity, has a low sensitivity for the detection of HNPGLs. Therefor its use for standard evaluation of HNPGLs is limited, and it is more frequently used to assess tumor avidity for the tracer if radionuclide therapy is planned (see 'other treatment'). In case of a patient that presents with catecholamine hypersecretion and multiple paragangliomas, ^{123}I-MIBG scintigraphy may also be used for the identification of the catecholamine producing paraganglioma. PET scanning can be used for the examination of the whole body and can detect small and metastatic lesions. ^{18}F-dihydroxyphenylalanine (^{18}F-DOPA) PET has a very good sensitivity for the detection of HNPGLs and is currently the functional imaging modality of choice in HNPGLs[36-38]. Because PGL and PCC overexpress somatostatin receptors (SSTRs), recent studies were able to show an excellent performance of ^{68}Ga-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-peptides in HNPGLs, and suggest a higher sensitivity and diagnostic value in the localization of HNPGLs. Furthermore, ^{68}Ga-DOTATATE PET-CT was shown to be superior for the detection of metastatic disease outside the head and neck area compared with other imaging modalities (^{123}I-MIBG, ^{18}F-DOPA-PET, CT or MRI) (see subheading '1.2.9 Malignancy')[39].

According to the Dutch guidelines for HNPGLs, all patients with at least one HNPGL are offered clinical surveillance and screening for plasma or 24-h urinary metanephrine (MN) or catecholamine concentrations[40]. Although some studies suggest plasma measurement has higher sensitivity and specificity, 24-h urine measurement of MNs has been shown to have sensitivity of up to 97% and specificity of up to 91% and is accepted as an alternative to the plasma test which has been reported to have sensitivity of 97% to 100% when performed correctly[41]. If catecholamine excess is demonstrated, an extensive workup is necessary to as-

sess the possibility of synchronous PCC or sPGL. Chromogranin A, a member of the granin family of neuroendocrine secretory proteins, is only rarely secreted and useful in the follow-up of selected tumors[42].

If a diagnosis of HNPGL is suspected, an incisional biopsy is contraindicated due to the risk of hemorrhage and subsequent fibrosis at the operative site[43]. Fine needle aspiration cytology (FNAC) is a simple, minimal invasive procedure in head and neck masses. Although the cytology features of PGL are not very specific and cytology alone is therefore not always sufficient for a reliable diagnosis of PGL, the FNAC technique has been found to be safe and is sometimes required in order to rule out other types of malignancy[44].

Macroscopically, PGLs are encapsulated, brownish tumors. PGLS typically appear to the surgeon as sharply circumscribed polypoid masses; they have a firm to rubbery consistency. Microscopically, PGLs are composed of clusters of epithelial cells in a highly vascular fibrous stroma (*Zellballen*; see also figure 1.2). Central necrosis or fibrous septa may be present. Extensive fibrosis may cause displacement and distortion of tumor nests with loss of the characteristic structure. Immunohistochemical staining shows positivity for S-100 protein, and a chromogranin stain for the cytoplasm of chief cells shows neurosecretory granules[45].

Treatment

In general, therapeutic options for HNPGL include surgical resection or debulking, radiotherapy, or active surveillance. The role of immunotherapy, peptide receptor radionuclide therapy (PRRT), and chemotherapy is subject of debate (see 'other treatment')[46]. The usual indolent growth pattern of HNPGL offers the opportunity for careful contemplation of a tailored-made treatment strategy. Optimal treatment is highly dependent on the tumor (location, size, involvement of neurovascular structures, malignancy and/or hypersecretion of catecholamines), the patient (age, comorbidities and symptoms) and the genetic status (implying potential for recurrence, malignancy or multicentric tumors; see subheading '1.3 Genetics of paragangliomas'). PGL care is highly multidisciplinary by nature, weighing potential risks and benefits of each treatment strategy per tumor site. The patients' preference plays an increasingly important role, especially in choosing between multiple valid treatment options.

Surgery

Complete surgical resection of HNPGL represents an curative treatment option and is considered in order to eliminate the (potential) source of catecholamine hypersecretion and/or to prevent morbidity associated with further tumor growth

or later spread from an unrecognized malignant tumor. In general, surgical success is measured by total tumor resection without recurrence (on imaging). Factors such as rapid growth, hypersecretion of catecholamines, aesthetic reasons, pain and/or concern for malignancy may support operative intervention. Contrariwise, advanced age, associated comorbidities and/or swallowing dysfunction may make surgery less advisable[47]. The surgical approach depends on the location of the tumor within the head and neck region, the extension of the tumor, and its relation to adjacent structures. Due to the high vascularity of HNPGLs and their close anatomical relationships with the carotid artery, the jugular vein, multiple cranial nerves and/or the skull base (figure 1.3), there is a definite risk of surgical complications. Important complications of surgery are vascular injury, cranial nerve injury, hypersecretion of catecholamines, and baroreflex failure.

Vascular injury

The occurrence of intraoperative or postoperative stroke (0-2%) has decreased dramatically as surgical and anesthetic techniques have improved[48-51]. This improvement has been attributed to many factors, including detailed preoperative imaging and angiographic evaluation to determine vessel involvement by tumor, carotid occlusion testing (see also 'diagnosis'), correlation of bilateral cerebral angiography findings with postocclusion cerebral function, and advances in surgical arterial revascularization techniques.

Cranial nerve injury

Surgical risk to the cranial nerves is site specific and related to tumor size. In general, the rate of postoperative cranial nerve dysfunction in HNPGL surgery ranges from 25 to 50%[49,52]. HNPGLs presenting with extensive skull base involvement are more likely to have lower cranial nerve involvement (cranial nerves IX-XII) and preoperatively cranial nerve deficits are often already present. Although isolated injury to one of the lower cranial nerves sometimes causes only temporary minor difficulty in swallowing, aspiration, phonation, shoulder mobility, or tongue motion, injury to the vagus nerve (X^{th} cranial nerve) and multiple cranial nerve injury may result in significant morbidity[53,54]. Familiarity with rehabilitation techniques is necessary for proper patient care. Injury to the spinal accessory nerve (XI^{th} cranial nerve) results in functional loss of the sternocleidomastoid and trapezius muscles. The majority of injured patients will benefit from referral to physical therapy avoiding shoulder pain secondary to limited range of motion. Hypoglossal nerve (XII^{th} cranial nerve) injury results in paresis or paralysis of the ipsilateral side of the tongue. Long-term paralysis may result in hemi-atrophy of this side of the tongue within a few months. If present, especially in combination with injuries to other lower cranial nerves, swallowing therapy may be necessary to prevent aspi-

ration. More significant, persistent aspiration and swallowing difficulties (particularly after injuries to the high vagus nerve) may require tracheostomy and feeding via a gastrostomy tube. Bilateral lower cranial nerve palsies generally represent a severe, potentially life-threatening situation (see subheading '1.2.6 Management of multiple and bilateral head and neck paragangliomas').

Hypersecretion of catecholamines

Surgical manipulation of HNPGL can lead to massive release of catecholamines ('*catecholamine storm*') and has the potential to cause hypertensive crisis, cardiac arrhythmias, myocardial ischemia, pulmonary edema, and stroke[55]. In order to avoid perioperative complications, systematic medical management is essential. Before the availability of pharmacological treatment in 1950s, the perioperative mortality was nearly 45% in adults[56]. However, the perioperative mortality has been reduced to less than 2% with appropriate blood pressure control[57]. The aim of adequate perioperative antihypertensive management is avoidance of fluctuation in blood pressure during surgical manipulation and prevention of postoperative hypotension resulting from the immediate decrease in catecholamine burden after tumor removal[41]. A sequential use of alpha-adrenergic receptor blockade and volume expansion followed by beta-blockade is recommended preoperatively to prevent blood pressure fluctuations. Postoperative hypotension is best avoided by achieving maximal vasodilation with judicious use of fluids and inotropic support[58].

Baroreflex failure

Resection of bilateral carotid body tumors can result in baroreceptive dysfunction. This dysfunction is due to bilateral denervation of the carotid sinus, manifesting as persistent tachycardia and hypertension (figure 1.3). Netterville *et al*. reported that 10 of 11 patients who underwent bilateral carotid sinus denervation demonstrated severe labile hypertension/hypotension, headache, diaphoresis, and emotional instability[59]. As the parasympathetic response is permanently lost, unopposed sympathetic stimuli can result in cardiovascular morbidity. This is usually successfully managed postoperatively with alpha-adrenergic antagonists. The long-term cardiovascular effects are controlled with clonidine or phenoxybenzamine (Dibenzyline).

Radiotherapy

The primary goal of treatment with radiotherapy in HNPGL is local tumor control by stopping further tumor progression. Radiotherapy can be used as a primary treatment or as an adjuvant therapy after surgical debulking. Currently the usual total dose is 45 gray[60]. This relatively low dose is sufficient to induce sub-

stantial sclerosis and fibrosis of tissue, and seems adequate in preventing tumor growth[61]. Higher doses are no longer used, except for the treatment of malignant tumors, although their response to radiotherapy seems to be very poor even using high doses[62]. While both conventional and stereotactic radiotherapy offer similar local control rates with acceptable toxicity, stereotactic radiotherapy has a favorable toxicity profile[63]. A definition of successful treatment with radiotherapy is difficult, as the natural course of most HNPGLs is characterized by no or slow growth. It is impossible to ascertain whether a non-growing HNPGL on imaging is the result of tumor control by successful radiotherapy or due to the indolent natural behavior of the disease[26,64]. In the literature, local tumor control after radiotherapy occurs in 88–100% of HNPGL cases with variable follow-up durations (50 months–11 years). The control rate decreases significantly with time: 95–96% at 5 years, 88–94% at 10 years, and 73% at 25 years. Complete tumor remission is extremely rare, but a slow reduction of tumor volume may occur[65-72]. The effect of radiotherapy on hypersecretion of catecholamines is as of yet not known. A few case reports have been published that suggest that catecholamine secretion from HNPGLs does not respond to radiotherapy[73]. Occasionally mild complications of radiotherapy (mucositis, nausea, fatigue, xerostomia and otitis) occur[65,67]. Especially in young patients, the most important concerns are those regarding serious late effects, i.e. brain or bone necrosis, although nowadays these serious sequelae appear to occur rarely (in 0.8 and 2.6% respectively)[72]. The radiation-induced malignancy rate is difficult to assess, due to different radiation techniques used and different follow-up durations. Aggressive osteosarcoma, fibrosarcoma and laryngeal carcinoma have been reported up to 25 years after treatment[10,74]. Radiotherapy may be considered as initial treatment modality especially for older patients with new cranial nerve deficits, whose risk of late recurrence or complications might exceed life expectancy, and those with bilateral large tumors and/or contraindications to surgery[75,76]. Salvage surgery after unsuccessful radiotherapy is sometimes indicated but generally technically difficult due to radiation-induced fibrosis.

Active surveillance

Whereas management of cervical PGLs with surgery and/or radiotherapy yields high rates of eradication or tumor control, these approaches may come with significant risk of short- and long-term morbidity as described above. Growing insight into the usually indolent natural course of HNPGLs has resulted in a relatively conservative approach of tumors (see also chapter 2 'Evolving management strategies'). This management strategy is called '*active surveillance'* (other terms such as '*watchful waiting'*, '*observation'*, or '*wait and scan*' are also widely used). It consists of regular monitoring of tumor progression with repeated imaging stud-

ies while no intervention is performed. Langerman *et al*. described 47 tumors (28 carotid body PGLs and 19 vagal PGLs) in 43 patients. During the study period, 42% tumors remained stable, 38% grew, and 20% regressed. Those that enlarged did so at a mean growth rate of 2 millimeter per year[77]. The main disadvantage of active surveillance is the risk of tumor progression and/or the development of new cranial nerve deficits, due to the close relationship of HNPGL with the lower cranial nerves[78]. New or worsening cranial neuropathies have been shown to develop in 11% to 33% of patients undergoing active surveillance[78-80]. If cranial nerve deficit occurs, it is usually better tolerated if the onset is slowly progressive due to tumor progression, as opposed to a sudden paralysis due to surgery. Based on these findings, the option of close observation may be considered for patients with limited symptoms, multiple tumors, elderly patients and patients with significant comorbidities. It is often the initial management option of choice in case of PGLs with high surgery- or radiotherapy-related risks.

Other treatments

The breakthrough of immunotherapy in the year 2013 has resulted in improved treatment of several cancers, including melanoma and lung cancer, and has demonstrated unprecedented, durable responses[81,82]. It appears that cancers with high mutation rates are particularly susceptible to the immune system. Although the genomes of PGL and PCC are relatively intact, and the mitotic index is characteristically low, the paraganglial cell is a dedicated entity with a unique set of transcripts. This finding could have a potential use for increasing immune cell recognition, either through already-registered immune checkpoint inhibitors (cytotoxic T-lymphocyte–associated antigen 4 (CTLA4) and programmed death 1 (PD-1) antibodies) or newer approaches, such as vaccines, immune cells, or microbe-based therapies[83,84]. PGL and PCC express SSTRs and hence PRRT with the use of DOTA-peptides is a promising treatment option. This treatment seems interesting for vagal PGL and larger jugulotympanic PGL that are rarely suitable for surgical removal. This is important, because therapeutic approaches for difficult to resect PGL are limited and most of these patients are not eligible for ^{131}I-MIBG treatment because of their lack of $^{123/131}$I-MIBG uptake (see also diagnosis). Puranik *et al.* described nine patients treated with PRRT using ^{90}Y/^{177}Lu-labelled peptides. In all patients PRRT was effective after positive confirmation of SSTR expression on ^{68}Ga-DOTATOC PET-CT, with no disease worsening seen, either in the form of neurological symptoms or distant spread. Though these are preliminary results, PRRT shows promising results and might be a good treatment option for HNPGL[85].
Chemotherapy has no role in the initial treatment of HNPGL. Chemotherapy with cyclophosphamide, vincristine, and dacarbazine can be used in patients who present with rapidly progressing metastatic disease[86].

Carotid body paragangliomas

Carotid body PGL is the most common HNPGL. The average age at diagnosis is 45 years and women are slightly more often affected than men. Characteristically carotid body PGLs present as a painless, slow growing neck mass. Hoarseness or dysphagia may be present in more advanced tumors. Clinical examination frequently demonstrates a pulsatile, lateral neck mass that is typically less mobile in the cephalocaudal direction due to adherence to the carotid artery, a finding known as a positive 'Fontaine's sign'. In up to 10% of carotid body PGL patients cranial nerve palsy is present, generally the vagus nerve[87]. Imaging shows a soft tissue tumor, seen at the level of the carotid bifurcation, characteristically splaying the internal and external carotid systems to create the 'lyre sign'. Carotid body PGLs can be classified according the Shamblin classification system, as described in 1971 (figure 1.4)[88].

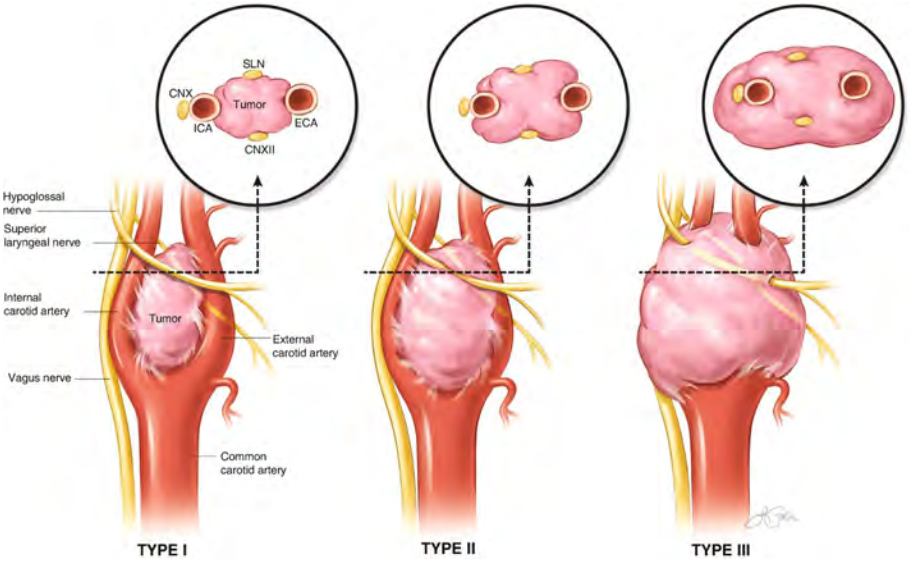


Figure 1.4 The classification of carotid body tumors according to Shamblin[88]. The top row shows the axial views; the bottom row shows the sagittal views of Shamblin type I, II and III paragangliomas. The classification is based on the relations of the tumor with the internal carotid artery (ICA), external carotid artery (ECA), vagus nerve (CNX), hypoglossal nerve (CNXII) and the superior laryngeal nerve (SLN). Adapted from Davis F.M., Obi A., Osborne N. (2018) Carotid Body Tumors. In: Hans S. (eds) Extracranial Carotid and Vertebral Artery Disease. Reprinted with permission by Springer.

The Shamblin classification is correlated with postoperative complications, intraoperative blood loss, and the need for vascular reconstruction, so early detection of carotid PGLs is important for safe management[89,90]. For smaller tumors (Shamblin type I and II) and for younger, healthy patients, surgical excision is considered the treatment of choice[91-93]. In case of larger tumors (Shamblin type III) and/or highly vascular tumors, there is a high risk of postoperative neurovascular complications. Therefore a vascular surgeon is an essential part of the surgical team as internal carotid artery injury occurs relatively frequently (10–23%) and reconstruction of the vessel leads to significantly lower stroke and mortality rates in comparison with ligation[89,94].
Early postoperative lower cranial nerve deficits, and Horner's syndrome (cervical sympathetic chain impairment), are relatively frequent complications(19–50%) [89,90,95]. In Shamblin type II and III carotid PGLs the rate of permanent neurological deficit is reported to be as high as 38%[96].

Vagal paragangliomas

Vagal PGLs originate from ganglia of the vagus nerve, usually from the nodose (inferior) ganglion[97]. Females are more often affected (female:male ratio is 1.87:1), with a mean age at diagnosis of 43 years[54]. The majority of patients with a vagal PGL do not have any symptoms (67%), and the tumor is identified coincidentally (as an incidentaloma) or through presymptomatic screening (chapter 6). The most frequently encountered symptom is a neck mass, followed by hoarseness. Vagal PGLs tend to occupy the post-styloid parapharyngeal space. On MRI the vagal PGL typically displaces the carotid system antero-medially. Several classification systems have been proposed, but none of these is universally accepted[98]. While surgical excision was traditionally the treatment of choice, it is now rarely recommended because of the associated morbidity. In most series, a postoperative vagus deficit by either injury or sacrifice of the nerve during surgery is almost universal (92–100%). This results in a unilateral vocal cord paralysis and pharyngeal plexus deficit (causing difficulty with speech and swallowing), along with ipsilateral pharyngeal numbness and velopharyngeal insufficiency[54,99-101]. Other new lower cranial nerve deficits occur postoperatively in 23–61% of the cases (mostly IXth cranial nerve), and in 15–17% of surgical cases the facial nerve (VIIth cranial nerve) is involved[99-102]. The majority of patients need complex rehabilitation management regarding speech, swallowing and facial nerve deficits (see also subheading 'Cranial nerve injury'). During follow-up these deficits may recover partially[54]. Compared to carotid PGLs, vagal PGLs are usually not as intimately associated with the great vessels, making vascular injury less likely[103]. An active surveillance strategy in vagal PGLs is associated with cranial nerve pal-

sies in only 7.5% of cases (vs 60% postoperatively in the same series) and a 5% increase in size had been observed over 8.5 years. Although 2.5% of these vagal PGL patients developed metastases during follow-up[101]. Because of the indolent behavior and the risk of postoperative cranial nerve deficits, active surveillance is considered the management option of choice for the vast majority of vagal PGL patients, especially the elderly, those with other/bilateral HNPGLs, and when swallowing or pulmonary pathology preexists. Radiotherapy can be considered in cases of tumor progression, with the aim of stabilization tumor growth[104].

Tympanic paragangliomas

Tympanic PGL is the most common primary neoplasm of the middle ear and the second most common tumor of the temporal bone[105]. These tumors are more common in the female population[105,106]. Typical presenting symptoms are conductive hearing loss and pulsatile tinnitus. Most tympanic PGLs are visible as a vascular middle ear mass (figure 1.5).

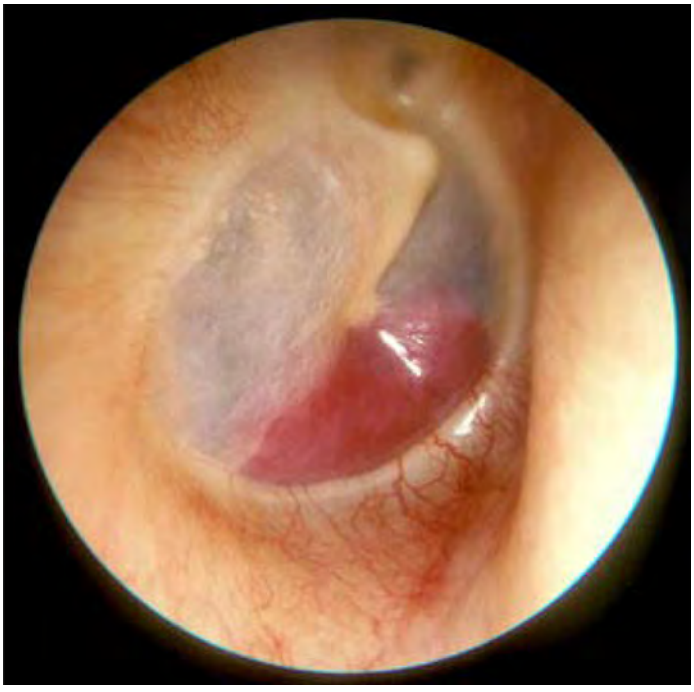


Figure 1.5 Red mass behind the right tympanic membrane, indicative of a tympanic paraganglioma (the 'rising sun' sign).

They are diagnosed by careful examination of the tympanic membrane and identification of the tumor through the translucent eardrum. Introducing positive pressure in the ear canal stops the pulsations of the tumor. Frequently, it is impossible to visualize the entire tumor clinically, thus CT or MRI scans are indispensable diagnostic tools. PGLs involving the temporal bone are generally classified according to the classification proposed by Fisch (table 1.1).

Table 1.1 The classification of temporal bone paraganglioma according to fisch[107].

Classification	Characteristic
Type A (tympanic paraganglioma)	limited to mesotympanum
Type B (hypotympanic paraganglioma)	limited to hypotympanum, mesotympanum, and mastoid without erosion of jugular bulb
Type C	involvement and destruction of infralabyrinthine and apical compartments
C1	no invasion of carotid canal; destruction of jugular bulb/foramen
C2	Invasion of vertical carotid canal between foramen and bend
C3	invasion along horizontal carotid canal
C4	invasion of foramen lacerum and along carotid canal into cavernous sinus
Type D	intracranial extension
De1	≤2 centimeter dural displacement
De2	>2 centimeter dural displacement
Di1	≤2 centimeter intradural extension
Di2	>2 centimeter intradural extension
Di3	inoperable intracranial invasion

The Fisch classification is primarily based on the extension of the tumor in the temporal bone and the involvement of the internal carotid artery, the jugular bulb, and the intracranial space. Fisch type A and B tumors are classified as tympanic PGLs.

Surgery is the main modality of treatment for tympanic PGLs. The tumor can be removed via a transcanal or postaural approach using bipolar electrocautery. In most series, treatment outcomes are reported together with jugulotympanic PGLs. However, surgery is much more straightforward and less complicated in purely tympanic PGLs. The results are expected to be favorable, probably because tympanic PGLs cause symptoms early and are diagnosed in less advanced stages. The gross total resection rate is 95–100%. Less than 8% of the patients show minor postoperative complications, and hearing is generally maintained or improved[105,108].

Jugulotympanic paragangliomas

Jugulotympanic PGL (Fisch type C and D tumors; table 1.1) typically present in the fifth and sixth decades of life and are three times more common in women. The growth rate of these tumors is generally slow (0.8 millimeter per year)[79]. Their indolent growth pattern makes it difficult to predict if and when these tumors will become clinically apparent; some tumors cause cranial nerve damage or invade the intracranial space, while others show spontaneous regression[109]. Jugular foramen PGLs may present with a variety of symptoms such as hearing loss, (pulsatile) tinnitus, dysphonia, shoulder weakness, dysarthria, and/or facial paralysis, due to involvement of the lower cranial nerves. Conductive hearing loss is seen with progression of the tumor into the tympanic space, which causes impairment of vibration of the ossicles. Sensorineural hearing loss and/or dizziness is reported by patients when the tumor has invaded the inner ear. Problems with swallowing and vocal cord function occur when cranial nerves IX and X are involved, however, these disease symptoms may be masked by compensation of function by the unaffected contralateral side. Intracranial extension may lead to compression of the brain and/or brainstem[110]. Physical examination may identify cranial nerve deficits and otoscopy may show a characteristic red, retrotympanic mass (figure 1.5). Irregular osseous erosion centered on the jugular foramen with further extension into the pneumatized spaces of the temporal bone is classically seen on CT imaging.

Surgery and radiotherapy for jugulotympanic PGL have a definite risk of cranial nerve damage or other serious adverse effects (see also 'Surgery' and 'Radiotherapy' in subheading '1.2.1 Head and neck paragangliomas'). Therefore, if clinical presentation does not require immediate therapy, an active surveillance strategy is the initial management of choice[111].

Studies that describe the experience with an active surveillance management for jugulotympanic PGL (excluding patients with brainstem compression or malignant disease) illustrated that only 20-60% of tumors showed further tumor growth and that additional treatment was required in only 0-5% of patients due to progression of existing cranial nerve damage[79,101,112,113]. Traditionally, surgery is considered the preferred treatment option if intervention is needed, as it actually removes tumor mass. However, recently radiotherapy has been advocated as it renders comparable local control rates and less iatrogenic cranial nerve damage or other complications such as cerebrospinal fluid leakage, wound infection or a stroke. Radiotherapy as a single modality results in excellent disease control (95%). New cranial nerve deficits were identified in 9.7%, 9.7%, 12%, and 8.7%

for cranial nerves IX, X, XI, and XII respectively[114]. Indications for debulking or resection may be young age, secreting tumors, significant intracranial mass effect, tumor progression (after radiation), facial paralysis and/or malignant transformation. Traditional surgical management through an infratemporal fossa approach entails closure of the external auditory canal and mobilization of the facial nerve, which results in a maximal conductive hearing loss and frequently a facial paresis. The gross tumor resection rate is around 40% in class D tumors and 35% in those with a large intradural extension (Fisch type Di2)[70,115,116].

The overall long-term tumor control has been reported to be 78.2% with a 1.6% treatment-related mortality rate. The risk of recurrence after apparent in toto resection is 6.9%[72]. In general, the functional outcome following surgery is poor. Immediate postoperative facial paresis is frequent and long-term dysfunction is present in 14–33% of the cases[70,117]. Up to 45.5% of the patients have some degree of hearing loss after surgery[72]. Other postoperative cranial nerve deficits for cranial nerves IX, X, XI and XII are 8%, 26%, 40% and 18% respectively[114]. Aspiration, infection and meningitis occur in less than 10% of the patients with possibly higher rates for a CSF leak (up to 14%)[72,75,100,118]. For tumors with significant intracranial extension as well as involvement of the middle ear and mastoid, a combined approach has been described where the jugulotympanic PGL is removed from the middle ear and mastoid while the remaining jugular foramen and intracranial component is treated with radiotherapy[119,120]. Critical neurovascular structures might be spared during surgery and if additional tumor growth is found with a consecutive wait-and-scan policy, radiotherapy could be applied. Although literature is sparse on this matter and sample sizes are small, combinations of surgery with Gamma Knife were described as a good alternative; local control was found in 80-100%, complications were found in 0-7%, and cranial nerve damage in 0-20% (11 months-7 years follow-up)[112,119-121].

Overall, for jugulotympanic PGLs, an initial wait-and-scan period should be considered. In the case of tumor growth (confirmed by imaging) or clinical progression of the tumor (indication of early cranial nerve palsy), radiotherapy might be the better option due to lower complication rates and similar or better local control rates when compared to surgery. It is important to acknowledge that the aims of these two treatment modalities are different, namely, eradication of tumor by surgery versus stabilization of tumor with radiotherapy. The most important aim of the therapy might however not be tumor eradication, but the best quality of life for the patient. In order to achieve that, the short and long-term sequelae of any therapy have to be weighed against the long-term natural behavior of the tumor.

Management of multiple and bilateral head and neck paragangliomas

In the case of bilateral HNPGLs, additional considerations apply. Frequently, an underlying genetic predisposition is present, putting these patients at higher risk of developing multiple synchronous or metachronous HNPGL, sPGL and/or PCC (see also subheading '1.3 Genetics of paragangliomas'). This may have important ramifications for treatment decisions in these patients, because bilateral cranial nerve involvement may result in significant impairment of speech, and difficulties in swallowing and breathing. If cranial nerve deficit occurs, it is usually better tolerated if the onset is slowly progressive, due to tumor progression, as opposed to a sudden paralysis due to surgery. Additional factors to consider include prior neck surgery or radiotherapy, patient's baseline cranial nerve function, life expectancy and pulmonary reserve.

In the management of bilateral HNPGL a dedicated multidisciplinary tumor team is essential and treatment options should be discussed with the patient, weighing potential risks and benefits of each treatment strategy per tumor site. When surgery is considered, it may be necessary to do so in a staged manner to diminish the risk of bilateral cranial deficits and/or impact on cerebral circulation. The choice of which side to treat first is a matter of debate, and as of yet there is no conclusive literature to guide clinicians. If difficulties are encountered during surgical resection of a PGL, the options of active surveillance or radiotherapy for the remaining tumor residue should be considered.

Management of functional head and neck paragangliomas

About one-third of HNPGL patients harbor catecholamine-hypersecreting tumors that may cause hypertension, paroxysmal palpitations, headache, agitation, excess sweating and/or pallor[22,24]. Prolonged exposure to high levels of catecholamines may eventually result in cardiovascular complications such as cardiac hypertrophy, myocardial infarction or heart failure. Multiple organ failure, shock and sudden death by stroke or cardiac arrest due to acute catecholamine excess have also been reported. Because of these potentially life-threatening conditions, surgical excision- if feasible- is the treatment of choice in functional PGLs (see also subheading 'Hypersecretion of catecholamines')[122-124].

Pheochromocytomas and sympathetic paragangliomas

Epidemiology

By definition, PCCs arise from the adrenal medulla whereas sPGLs arise from extra-adrenal paraganglia, with a predilection for the mediastinum (from the thoracic sympathetic chain) and the abdominal and pelvic para-aortic regions. The incidence of PCC is 2-8 per million persons per year[125,126]. PCC is present in 0.1-1% of patients with hypertension[127-129]. The peak incidence occurs in the third to fifth decades of life; the average age at diagnosis is 24.9 years in hereditary cases and 43.9 years in sporadic cases[130]. The incidence is equal for males and females[131].

Signs and symptoms

The clinical presentation of sPGL and PCC is variable due to different profiles of catecholamines secreted, desensitization of adrenoreceptors (most likely due to long-term exposure to high circulating catecholamine levels), and presentation of symptoms related to tumor bulk[132]. Therefore, sPGL/PCC is also called 'the great masquerader'. Hypertension, continuous or paroxysmal, is the most common feature of advanced sPGL and PCC. Typical symptoms are paroxysms of severe headache, palpitations, and diaphoresis, 'the classic triad'. Paroxysms can last minutes to hours, with varying intervals, and occur spontaneously or be triggered by direct stimulation of the tumor (e.g. micturition in case of a bladder localization), physical activity, diagnostic procedures, or certain drugs (e.g. metoclopramide, glucagon, and glucocorticoids)[133,134]. Other symptoms may include anxiety, nausea, vomiting, and weakness[135].

Diagnosis

Clinical suspicion should be followed by biochemical testing to rule out the potentially lethal catecholamine excess and to diagnose sPGL or PCC. The biochemical diagnosis consists of demonstration of hypersecretion of catecholamines (epinephrine, norepinephrine, and dopamine) or their metabolites (metanephrine (MN), normetanephrine (NMN), and 3-methoxytyramine (3-MT) respectively)[136]. After establishing a biochemical diagnosis, sPGL/PCC can be localized and staged by anatomical and functional imaging studies. Anatomical imaging (CT or MRI) has an excellent sensitivity (77–98 and 90–100% respectively) but lacks specificity (29–92 and 50–100% respectively) for detecting sPGL/PCC[137,138]. Tumors detected by anatomical imaging can subsequently be identified as PGL/PCC by functional imaging agents that specifically targets the catecholamine synthesis, storage, and secretion pathway of chromaffin cells. ^{123}I- or ^{131}I-MIBG scintigraphy is the most widely available and used nuclear imaging technique in the

initial functional imaging of PGL/PCC. ^{18}F-DOPA-PET has been demonstrated to be useful in the evaluation of sPGL and HNPGL[139]. ^{68}Ga-DOTATATE PET-CT has also been advocated due to the higher lesion to background tissue contrast and high specificity for PCC[140].

Treatment

The treatment of choice for sPGL and PCC is surgical resection, preferably laparoscopically, but in case of a large tumor (in general >6 cm) with a higher risk of malignancy, conventional laparotomy is performed[141]. In order to minimize surgical complications (hypertensive crisis and arrhythmias), adequate pretreatment is necessary, consisting of alpha-blockade (doxazosin and phenoxybenzamin) titrated at orthostatic hypotension, followed if needed by addition of beta-blockade (propanolol and atenolol), especially in case of tachycardia.

Malignancy

Benign and malignant PGL have a similar histology, and it is extremely difficult for pathologists to differentiate between the two. Therefore, malignancy is defined by the presence of metastases: PGL tissue at sites where chromaffin tissue is normally absent[142,143]. Nearly 10% of PCC and 10–20% of sPGL are malignant, whereas HNPGLs are usually benign[144,145]. Malignant HNPGLs usually present with regional metastases in cervical lymph nodes or systemic metastases, usually to bones, lung, and liver. Metastatic disease is frequently associated with pathogenic variants in succinate dehydrogenase subunit B (*SDHB)* (see subheading '1.3 Genetics of paragangliomas')[146-148]. For the evaluation of suspected metastatic PGL, ^{18}F-fluoro-2-deoxyglucose (FDG) PET is recommended (sensitivity 74–100%), with the highest sensitivity for metastatic *SDHB*-related PGL/ PCC[139,149]. In addition, ^{111}In-pentetreotide scintigraphy may be useful in detecting MIBG-negative metastases[137]. ^{68}Ga-DOTATATE PET-CT is superior for the detection of metastatic disease outside the head and neck area than other imaging modalities (^{123}I-MIBG, ^{18}F-DOPA-PET, CT or MRI)[39]. The primary management of patients with malignant PGL should be directed toward complete surgical resection of the primary tumor and regional lymph nodes. Postoperative radiation may be beneficial in slowing the progression of residual disease[145]. Systemic treatment options include radionuclide therapy with ^{131}I-MIBG or radiolabeled somatostatin analogues, however ^{131}I-MIBG has proven to be the most effective non-surgical therapeutic modality[150]. Other treatment options are peptide receptor radionuclide therapy (PRRT) using radiolabeled somatostatin analogues like ^{177}Lutetium-DOTA-octreotide and ^{90}Yttrium-DOTA-lanreotide[151]. More recently, studies assessing targeted therapies, such as Sunitinib, have shown promising results in the treatment of malignant

PGL/PCC[152,153]. Sunitinib is an oral tyrosine kinase inhibitor with antiangiogenic and antitumor activity.

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%[144]. Survival seems to be influenced by the causative gene, as the 5-year survival rate after first metastasis is 36% in patients carrying a variant in the *SDHB* gene, whereas it is 67% in the absence of *SDHB* variants[154].

1.3 GENETICS OF PARAGANGLIOMAS

PGL and PCC show the highest level of hereditability (approximately 40%) of all human tumors, and around two thirds of hereditary cases are accounted for by pathogenic variants in genes encoding subunits or cofactors of the succinate dehydrogenase (*SDH*), the first metabolic enzyme known to act as a tumor suppressor. The first of this group of PGL susceptibility genes to be discovered was *SDHD,* almost two decades ago in the year 2000[155,156].

The Cancer Genome Atlas (TCGA) proposes that PCC and PGL can be divided into three main molecular subgroups that have been linked to distinct driver genes:

1. *Pseudohypoxia.* The pseudohypoxia group can be divided into at least two subgroups: tricarboxylic acid (TCA) cycle-related genes, containing the genes encoding *SDH* subunits *SDHA*, *SDHB*, *SDHC* and *SDHD,* as well as *SDHAF2* (*SDHx*), an assembly factor of the *SDH* complex, and *FH*, a second enzyme in the TCA cycle; and *VHL*/*EPAS1*-related, with somatic and germline mutations. Mutations in genes that are involved in the pseudohypoxic pathway result in a significant increase in vascularization and in the expression of vascular endothelial growth factor (VEGF) and its receptors. In addition, some members of the group have impaired DNA demethylation.
2. *Wnt-altered.* The Wnt gene family encodes a set of highly conserved secreted signaling proteins that have major roles in embryogenesis and tissue homeostasis. The Wnt signaling group includes newly recognized somatic mutations in *CSDE1* as well as somatic gene fusions affecting *MAML3*.
3. *Kinase signaling*. The kinase signaling group consists of germline or somatic mutations in *RET*, *NF1*, *TMEM127*, *MAX*, and *HRAS*[157-159].

Each subgroup has a unique phenotype, which can be used to personalize care; precision medicine and targeted therapies[158,160].

Table 1.2 Paraganglioma and pheochromocytoma genes and genetic syndromes[172].

Gene	Syndrome	Transmission	Penetrance HNPGL	Penetrance sPGL	Penetrance PCC	Other manifestations
SDHA	Familial PGL type 5	AD	Very low	Very low	Very low	GIST, pituitary tumors
SDHB	Familial PGL type 4	AD	Intermediate	Intermediate	Low	GIST, pituitary tumors, and RCC
SDHC[a]	Familial PGL type 3	AD	Low	Low	Low	GIST, RCC
SDHD	Familial PGL type 1	AD, paternal	High (multifocal)	Low	Low	GIST, pituitary tumors, and RCC
SDHAF2	Familial PGL type 2	AD	High (multifocal)	Very low	Very low	
FH	Hereditary leiomyomatosis and renal cell cancer[b]	AD	Unknown	Unknown	Unknown	Leiomyomatosis, RCC
VHL	Von Hippel-Lindau syndrome	AD	Very low	Low	High (bilateral)[c]	Hemangioblastoma, RCC, epididymal cystadenoma, pancreatic neuroendocrine tumors, retinal abnormalities
EPAS1	PGL-PCC-somatostatinoma-polycythemia syndrome (Pacak-Zhuang dyndrome)	Unknown (postzygotic)	Very low	High (multifocal)	High	Polycythemia, somatostatinoma, retinal abnormalities, organ cysts
CSDE1	No predisposition to PGL-PCC (only somatic mutations reported)					
MAML3	No predisposition to PGL-PCC (only somatic mutations reported)					
RET	Multipele endocrine neoplasia type 2	AD	Very low	Very low	High (bilateral)[d]	MEN2A: medullary thyroid carcinoma, parathyroid adenoma, MEN2B: medullary thyroid carcinoma, mucosal neuromas, dysmorphic features

Table 1.2 Continued

Gene	Syndrome	Transmission	Penetrance HNPGL	Penetrance sPGL	Penetrance PCC	Other manifestations
NF1	Neurofibromatosis type 1	AD	Very low	Very low	Low (bilateral)	Café-au-lait-spots, Lisch nodules in the eye, neurofibromas, intellectual disability, dysmorphic features, skeletal abdormalities
MAX	Familial PGL-PCC, MAX-related	AD	Unknown	Unknown	Unknown	Renal oncocytoma
TMEM127	Familial PGL-PCC, TMEM127-related	AD	Very low	Low	Intermediate	
HRAS[e]	No predisposition to PGL-PCC (only somatic mutations reported)					

Patients with familial paraganglioma and pheochromocytoma related to SDHA-SDHD variants may present with Carney Stratakis syndrome (PGL and GIST) or Carney triad (PGL, GIST and pulmonary chondroma).

Abbreviations: PGL, paraganglioma; PCC, pheochromocytoma; AD, autosomal dominant; GIST, gastrointestinal stromal tumor; MEN2, multiple endocrine neoplasia type 2; RCC, renal cell carcinoma.

[a] Occurs both as genomic and epigenomic mutations.

[b] Paraganglioma and pheochromocytoma not included in clinical criteria.

[c] High in VHL type 2, low in VHL type 1.

[d] Genotype dependent, 50% in cysteine mutations, low in noncysteine sites.

[e] Constitutional mutations in HRAS codons 12, and rarely 13, cause Costello syndrome. To our knowledge no cases of PGL-PCC and germline HRAS mutation have been described in the literature. It should be noted that somatic HRAS mutations in PGL-PCC are restricted to codon 61 and rarely codon 13.

Worldwide, variants in the *SDHB* gene account for 10% of cases of all PGL/PCC and approximately one quarter of familial disease[161-163]. Variants in the *SDHD*, *SDHA* and *SDHC* gene account for approximately 5-9%, 1% and 1-2% respectively of cases of PGL/PCC[161,162]. Very few cases of PGL/PCC associated with variants in *SDHAF2* have been described and account for <0.1% of cases of all PGL/PCC[164,165]. In the Netherlands, pathogenic variants in *SDHD* are the most prevalent cause of PGL syndrome, followed by variants in *SDHB* and *SDHA*[166,167]. Although all *SDHx* genes encode subunits of the same SDH complex and pathogenic variants all disrupt its enzymatic function, different *SDH* genes are associated with different phenotypes. *SDHD* mutation carriers have a significant risk of developing multiple HNPGLs, with a low incidence of malignancy. *SDHB* mutation carriers are reported to develop solitary PGLs and metastatic PGLs more frequently (chapter 5). Compared to the general population, mortality seems to be increased in *SDHB* variant carriers, especially in those affected by PGL. In *SDHD* variant carriers, the mortality is comparable to that of the general Dutch population, even if they are affected by PGL (chapter 8).

Mutations of *VHL*, *RET*, and *NF1* occur predominately in patients suffering from PCC and are rare in HNPGL, whereas mutations of *SDHB* and *SDHD* are common in PGL patients but uncommon in solitary PCC[168]. Some of the genes responsible for PGL and PCC are linked to other tumor types and clinical syndromes. The classical syndromes include neurofibromatosis type 1 (NF1), multiple neuroendocrine neoplasia (MEN) syndrome type 2, and von Hippel-Lindau syndrome (VHL). Also, mutations in *SDH* genes contribute to the understanding of hereditary PGL-PCC syndromes, Carney's triad, and Carney-Stratakis syndrome. Conversely, hereditary PGL or PCC associated with *TMEM127* or *KIF1B* mutations are not syndromic[169,170].

In table 1.2 the different genes associated with PGL/PCC and PGL syndromes are displayed. In *SDHD*, *SDHAF2*, and *MAX* linked cases transmission of hereditary PGL or PCC is subject to parent-of-origin dependent inheritance. The disease generally manifests only following paternal transmission of the disease gene[171].

Associated non-paraganglionic tumors

SDHx mutations have also been linked to non-paraganglionic tumors (see table 1.2 'Other manifestations'). *SDHB*-related renal cell carcinomas (RCC) have been described (chapter 5). *SDH*-related RCCs have distinct clear cell pathological features and are acknowledged as a unique subtype of RCC[173]. They appear to occur at a younger age and are characterized by a more aggressive behavior than their sporadic counterparts[148,155,173-176]. It has been hypothesized that there may be certain *SDHB* pathogenic variants that increase the risk of devel-

oping a renal tumor; those with arginine substitutes appear particularly predisposed, although other genotype associations have also been reported[173,177]. Gastrointestinal stromal tumors (GIST) are reported to occur in approximately 2% of *SDHB* variant carriers and also a predisposition to developing pituitary adenomas has been found in the association with *SDHB* pathogenic variants[178,179]. Very few RCC cases have been reported in *SDHD* variant carriers and lifetime risk is low (<1%)[177]. GIST and pituitary adenoma have been described in patients carrying *SDHA* variants with PGL/PCC disease, and also metastatic GIST has been reported[180]. Remarkably, approximately 50% of *SDH*-deficient GIST are due to somatic mutations, and *SDH*-deficient GIST are now recognized as a unique class of GIST[181,182]. Finally, pancreatic neuroendocrine tumors may also be part of the *SDH*-related tumor spectrum[183].

1.4 PENETRANCE

Definition

Penetrance in genetics is the proportion of individuals carrying a particular gene variant (the genotype) that also express an associated trait (the phenotype). In medical genetics, the penetrance of a disease-causing variant is the proportion of individuals with the variant who exhibit the associated clinical disease. Accurate estimates of this age-dependent disease risk are important in counseling patients and their families and in optimizing cascade screening and follow-up protocols (surveillance).

SDHA

To our knowledge, no PGL family-based studies have been reported in *SDHA* variant carriers and the penetrance therefore remains unknown, but is likely to be low. In a multicenter cohort, the estimated penetrance of any *SDHA*-related manifestation was 10% at age 70 years in (non-index) variant carriers[167].

SDHB

The reported penetrance of *SDHB* variants varies widely (9%-75%). While initial penetrance estimates were high, over time lower estimates have been reported due to the inclusion of more disease-free asymptomatic carriers in penetrance calculations and improved calculation methodology (see chapter 4 and 7). The overall penetrance of *SDHB* variant carriers is now estimated to be 21% at age 50 and 42% at age 70 when adequately corrected for ascertainment. Similar disease risks are found for different *SDHB* variants as well as for male and female *SDHB* variant carriers (chapter 7).

SDHC

The estimated risk of developing PGL/PCC at age 60 in *SDHC* variant carriers is 25%[156].

SDHD

The lifetime penetrance for *SDHD* variant carriers has been reported to be very high (88-100%), and approximately 75% of carriers will manifest disease by age 40[148,184-186].

SDHAF2

To date, only 60 cases have been described including at least two different families. Eighty-six percent of the investigated variant carriers had disease, the majority of whom had multiple HNPGLs[179,187].

Penetrance calculations*

Increased cascade screening is leading to the identification of increasing numbers of, mostly asymptomatic, pathogenic gene variant carriers, family members of index patients. This results in more accurate estimates of disease-risks associated with the pathogenic gene variant.

Different family-based study designs have been suggested for penetrance estimations[188]. A frequently used method for the estimation of the penetrance uses the Kaplan-Meier estimator based on data of the relatives of index patients only (the index patients themselves are left out of the analysis to correct for the ascertainment bias to prevent overestimation of the penetrance). This method may still yield biased estimates as it does not actually correct for the way the data are ascertained. Moreover, leaving out the index patients from the analysis means discarding valuable information, especially in rare and low-penetrant disease.

In low-penetrant disease, essential pedigree information is usually missing due to a plethora of possible reasons: the patient may be unaware of the family history, the hereditary nature of the disease may not have been recognized during treatment of a seemingly sporadic patient, or the data were initially not collected for research or cascade screening purposes. Usually, pedigree data are obtained via an index patient in a pedigree. Patients who express the disease phenotype and

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carry the genetic variant of interest are asked to inform their family members about their potential risk. Some of these relatives will consent to genetic counseling and DNA testing. Detected carriers of the germline variant will be screened for the disease. When aiming to estimate the penetrance of the disease, the follow-up data of all known carriers (the index patients and their relatives carrying the gene variant) are collected from the medical records. However, the relation between the carriers and the index patient is often unclear. This missing information hampers the correction for the way the data were ascertained. In the appendix of chapter 7 we describe a novel method for the estimation of the penetrance function that is designed especially for the situation described above.

1.5 OUTLINE OF THE THESIS

Variants in different PGL genes are surprisingly different in terms of inheritance, penetrance, tumor location, risk of malignant transformation and mortality. The aim of this thesis is to gain insight in the clinical consequences for PGL patients, with a focus on carriers of a pathogenic *SDHB* variant.

Chapter one is an introduction to the paraganglion system, the current insights in HNPGL, sPGL and PCC, the diagnosis and treatment, the causative genes and their phenotypes, the heredity and penetrance of PGL syndromes.

In **chapter two**, the clinical characteristics of HNPGL patients treated at the Amsterdam University Medical Center, location VUmc, are evaluated. It describes the changing management strategies in HNPGL patients over six decades (1956-2015).

Chapter three describes a novel *SDHB* germline variant associated with HNPGL in a Dutch kindred.

In **chapter four** the phenotype of the exon 3 deletion in *SDHB* is studied in a large multigenerational PGL family, with a focus on the penetrance of this specific variant.

In **chapter five** the phenotypical characteristics of a nationwide cohort of *SDHB* germline mutation carriers are evaluated and differences in clinical phenotypes related to specific *SDHB* mutations are assessed.

Chapter six reports on clinical characteristics and outcome of treatment strategies for patients with HNPGL carrying *SDHB* germline mutations.

In **chapter seven** the penetrance of PGL and PCC in *SDHB* germline mutation carriers is calculated in a nationwide cohort, using a novel maximum likelihood estimator. This estimator addresses ascertainment bias and missing data on pedigree size and structure.

In **chapter eight** the mortality of a nationwide cohort of *SDHB* variant carriers and that of a large cohort of *SDHD* variant carriers is estimated and compared to the mortality of a matched cohort of the general Dutch population.

Chapter nine consists of a summary of the thesis, its general implications for patients carrying a mutation in *SDHB* and future perspectives of PGL research.

1.6 ABBREVIATIONS

3-MT	3-methoxytyramine
CT	computed tomography
CTA	computed tomography angiography
DOPA	dihydroxyphenylalanine
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
DSA	digital subtraction angiography
ECA	external carotid artery
FDG	fluorodeoxyglucose
FNAC	fine needle aspiration cytology
GIST	gastrointestinal stromal tumors
HNPGL	head and neck paraganglioma
ICA	internal carotid artery
MEN	multiple neuroendocrine neoplasia
MIBG	metaiodobenzylguanidine
MN	metanephrine
MRA	magnetic resonance angiography
MRI	magnetic resonance imaging
NF1	neurofibromatosis type 1
NMN	normetanephrine
PCC	pheochromocytoma
PET	positron emission tomography
PGL	paraganglioma
PRRT	peptide receptor radionuclide therapy
RCC	renal cell carcinoma
SDH	succinate dehydrogenase
SDHA	succinate dehydrogenase subunit A
SDHAF2	succinate dehydrogenase assembly factor 2
SDHB	succinate dehydrogenase subunit B
SDHC	succinate dehydrogenase subunit C
SDHD	succinate dehydrogenase subunit D
SSTR	somatostatin receptor
TCA	tricarboxylic acid (cycle), or Krebs cycle
TCGA	The Cancer Genome Atlas
TMEM127	transmembrane protein 127, may refer to gene or protein
VEGF	vascular endothelial growth factor
VHL	von Hippel-Lindau syndrome, may refer to the VHL syndrome, gene or protein

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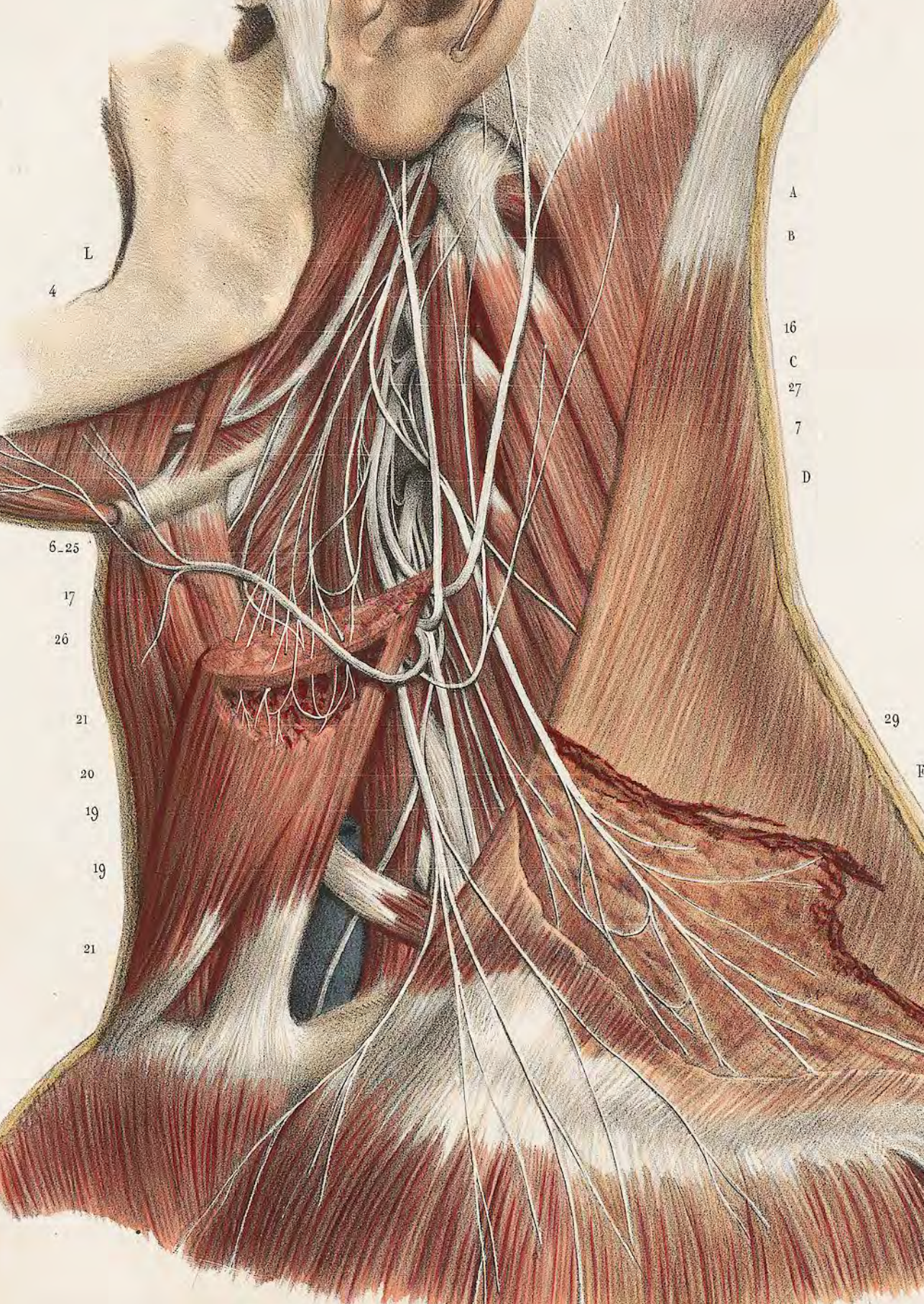
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2 Evolving management strategies in head and neck paragangliomas: A single-centre experience with 147 patients over a 60-year period

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2.1 INTRODUCTION

Paragangliomas (PGLs) are rare, slow-growing and usually benign tumours that arise in the paraganglion tissue associated with the autonomic nervous system. PGLs can be divided into head and neck paragangliomas (HNPGLs), sympathetic paragangliomas (sPGLs) located in the abdomen or thorax, and pheochromocytomas (PHEOs) located in the adrenal glands. Generally, HNPGLs are of parasympathetic origin and about one-third of HNPGL patients have catecholamine-secreting tumours that may cause elevated blood pressure, palpitations, flushes and agitation[1].

Head and neck paragangliomas most frequently originate from the paraganglia in the bifurcation of the carotid artery, the jugular foramen, along the vagal nerve or along the tympanic nerve. Rarely, HNPGLs are located elsewhere in the head and neck region, that is, the nasal cavity, paranasal sinuses, parotid gland, cervical sympathetic chain, pharynx, larynx, trachea, aortic arch, ciliary ganglion and thyroid gland[2]. Most HNPGLs are characterised by slow and expansive growth, but approximately 10%-15% of the tumours show a more aggressive, rapidly progressive behavior[3]. Symptoms vary with the tumour localisation and the associated cranial nerve deficits.

Head and neck paraganglioma can occur sporadically or as part of a hereditary syndrome. PGL syndromes are mainly caused by germline mutations in genes encoding subunits or cofactors of the mitochondrial succinate dehydrogenase (*SDH*), respectively, *SDHA*, *SDHAF2*, *SDHB SDHC* and *SDHD*. An increasing number of other genes have been associated with the development of PGL, for example *RET*, *NF1*, *VHL*, *HIF2A*, *FH*, *TMEM127* or *MAX*. Different causative genes are associated with different clinical characteristics[4]. In the Netherlands, pathogenic variants in *SDHD* are the most prevalent cause of PGL syndrome, followed by variants in *SDHB* and *SDHA*[5,6]. *SDHD* mutation carriers have a significant risk of developing multiple HNPGLs, with a low incidence of malignancy (1.7%). *SDHB* mutation carriers are reported to develop solitary PGLs and metastatic PGLs more frequently (7.3%)[7]. In this study, we evaluated clinical characteristics and treatment strategies of 147 consecutive patients with a total of 289 HNPGLs referred to the department of Otolaryngology/Head and Neck surgery of the Amsterdam University Medical Centres, Vrije Universiteit Amsterdam, the Netherlands, during the last 60 years.

2.2 MATERIALS AND METHODS

Patients visiting the department between 1956 and 2017, with at least one HNPGL were included. Patient characteristics including genetic status (if available), gender, family history, age at diagnosis, number and localisation of HNPGLs, concurrent sPGL, PHEO, metastatic disease, management strategy and outcome were recorded. The duration of follow-up was defined as the period between the date of HNPGL diagnosis (on imaging) and the most recent outpatient clinic visit. The diagnosis of HNPGL was based on patient and family history, otolaryngology examination including otoscopy and laryngoscopy, and/or computed tomography (CT) imaging, and/or magnetic resonance (MR) imaging and/or an angiography of the head and neck region including the skull base. Since 2003, HNPGL patients (and family members at risk) have been offered genetic counselling and DNA testing. Biochemical screening including the measurement of (nor)adrenaline, vanillylmandelic acid (VMA), dopamine, (nor) metanephrine and/or 3-methoxytyramine (3-MT) in two 24-h urinary samples and/or plasma-free (nor)metanephrine was offered to HNPGL patients. In case of excessive catecholamine secretion, additional radiological assessment by MR imaging or CT scans of thorax, abdomen and pelvis and/or ^{123}I-metaiodobenzylguanidine (MIBG)-scan, and/or 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. In *SDHB* mutation carriers, MR imaging of the thorax, abdomen and pelvis was performed as standard routine. Active surveillance (also called “wait and scan policy” or “watchful waiting”), radiotherapy, surgical resection or combinations were possible treatment strategies and were multidisciplinary discussed, weighing potential risks and benefits of each treatment strategy per tumour and per patient. Active surveillance, and postoperative and post-irradiation follow-up comprised of regular MR imaging and clinical evaluation by an endocrinologist and ENT surgeon. The interval was determined by several factors, such as tumour size, tumour progression rate, tumour localisation, symptoms and treatment modality, and thus differed per tumour and per patient. IBM SPSS Statistics version 20.0 (SPSS) was used for data analysis.

Ethics approval and consent to participate

The study was approved by the institutional Medical Ethics Committee (VUMC; number 2017.238). The authors declare that all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration. For this type of study, formal consent is not required.

2.3 RESULTS

Clinical characteristics

One hundred and forty-seven patients, 47 male (32%) and 100 female (68%), with a total of 289 HNPGLs were diagnosed in a 60-year period. Sixty-three patients (43%) presented with a positive family history, while the remaining 84 patients (57%) had no known family history of (HN)PGL or PHEO. The mean age at diagnosis was 45.3 years (95% CI: 42.5-48.0) and ranged from 11 to 88 years. The mean duration of follow-up was 13.1 years (range 0.03-60.9, median 8.9). Four HNPGL patients (3%) developed a PHEO and two patients (1%) a sPGL. The vast majority of HNPGLs (286/289; 99%) was located at the bifurcation of the carotid artery (127/289 tumours; 44%, in 87 patients), the jugular foramen (68/289 tumours; 24%, in 63 patients), along the vagal nerve (58/289 tumours; 20% in 51 patients) or along the tympanic nerve (33/289 tumours; 11% in 32 patients). Other locations were the larynx, pharynx and nasal cavity (3/289 tumours; 1%, in three patients), and these tumours were confirmed to be PGL by histopathology. Multiple synchronous or metachronous HNPGLs were found in 79 of 147 patients (54%), up to a maximum of six metachronous HNPGL.

At diagnosis, 29 out of 96 (30%) biochemically screened HNPGL patients showed excessive catecholamine secretion. In 25 out of 29 (86%) of these patients, additional imaging was performed in order to identify the source of catecholamine excess. Two of these patients (2/29; 7%) were diagnosed with a concurrent PHEO, one of these patients was diagnosed with metastatic disease (1/29; 3%), and 2 (2/29; 7%) patients were diagnosed with a sPGL. This percentage was 6/10 (60%) for *SDHB* patients and 16/52 (31%) for *SDHD* HNPGL patients. In three of four patients (75%) with a concurrent PHEO, excessive catecholamine secretion was present.
DNA tests were performed in 98/147 (67%) of HNPGL patients. Patient characteristics categorised per genetic subgroup are outlined in Table 2.1.

Table 2.1 Characteristics of 98 DNA-tested HNPGL patients

Patient characteristics	*SDHD* pathogenic variant (n = 64; 65%)	*SDHB* pathogenic variant (n = 10; 10%)	*SDHAF2* pathogenic variant (n = 1; 1%)	No *SDHx* pathogenic variant (n = 23; 23%)
Male/ female	20/44	4/6	0/1	7/16
Mean age at diagnosis (95% CI)	38.2 (34.9-41.4)	45.6 (35.9-55.3)	15	56.6 (50.7-62.5)
Metastatic disease	3 (5%)	-	-	-
Multiple HNPGL	56 (88%)	2 (20%)	-	2 (7%)
PHEO	4 (6%)	-	-	-
sPGL	2 (3%)	-	-	-

HNPGL: head and neck paraganglioma; *PHEO*: pheochromocytoma; *sPGL*: sympathetic paraganglioma.

Table 2.2 Clinical characteristics, treatment strategies and outcome of head and neck paraganglioma patients with metastatic disease

Case	Sex	Mutation	Location HNPGL	Age[a]	Age[b]	Location metastases (age)	PHEO	Treatment HNPGL (age)	Treatment malignant disease (age)	Outcome
1	F	*SDHD* p.Asp92Tyr	CBPL JFPR VPR	34	37	Paravertebral T7 (37) Mediastinal (47) Pulmonary (47) Cardial (53)	Yes	surgery VPR, JFPR (35) RT CBPL (37) RT VPR, JFPR (37)	RT paravertebral T7 (37)	Alive at age 53, with disease
2	M	*SDHD* p.Asp92Tyr	JFPR	39	47	Mediastinal	Yes	No	No	Alive at age 48, with disease
3	F	*SDHD* p.Asp92Tyr	CBPR CBPL	45	64	Bone (vertebra)	No	surgery CBPL, CBPR (45)	anterior corporectomy C3-5 and partially C6 (64) Lu-177-ocreotide therapy (65) Lu-177-ocreotide therapy (72)	Alive at age 75, with disease

a: age in years at diagnosis of head and neck paraganglioma.
b: age in years at diagnosis of metastatic disease.
M: male; *F*: female; *HNPGL*: head and neck paraganglioma; *JFPR*: jugular foramen paraganglioma right; *CBPL*: carotid body paraganglioma left; *CBPR:* carotid body paraganglioma right; *VPR:* vagal paraganglioma right; *RT:* radiotherapy.

Table 2.3 Overall treatment strategy and outcome in patients with a solitary head and neck paraganglioma

Tumour localisation	Overall outcome (%)	Mean follow-up (years)	Treatment strategy	Treatment strategy n (%)	Tumour classification[a]							Mean follow-up (years)	Out come		
													NED	*AWD*	*DID*
					Shamblin										
					Type 1		***Type 2***		***Type 3***		***Unknown[b]***				
Carotid body tumour (n = 17)	NED 8 (47%) AWD 9 (39%) DOD 0 DID 0	11.2	Active surveillance Surgery Radiotherapy Surgery + adjuvant radiotherapy	8 (47%) 9 (53%) - -	- 2 - -		5 4 - -		3 2 - -		- 1 - -	7.7 14.3 - -	- 8 (89%) - -	8 (100%) 1 (11%) - -	- - - -
					Fisch										
					Type C1	***Type C2***	***Type De1***	***Type De2***	***Type Di1***	***Type Di2***	***Unknown[b]***				
Jugular body tumour (n = 25)	NED 3 (12%) AWD 19 (76%) DOD 0 DID 3 (12%)	8.7	Active surveillance Surgery Radiotherapy Surgery + adjuvant radiotherapy	12 (48%) 8 (32%) 3 (12%) 2 (8%)	10 - 2 -	- - 1 1	- 1 - -	- - - -	- - - 1	1 - - -	1 7 - -	6.3 9.2 9.8 19.9	- 2 (25%) - -	11 (92%) 4 (50%) 3 (100%) 2 (100%)	1 (8%) 2 (25%) - -
Vagal body tumour (n = 10)	NED 2 (20%) AWD 8 (80%) DOD 0 DID 0	7.2	Active surveillance Surgery Radiotherapy Surgery + adjuvant radiotherapy	5 (50%) 4 (40%) 1 (10%) -								2.2 14.2 4.6 -	- 2 (50%) - -	5 (100%) 2 (50%) 1 (100%) -	- - - -
					Fisch										
					Type A		***Type B***		***Unknown[b]***						
Tympanic body tumour (n = 15)	NED 10 (66%) AWD 5 (33%) DOD 0 DID 0	3.1	Active surveillance Surgery Radiotherapy Surgery + adjuvant radiotherapy	5 (33%) 10 (67%) - -	2 3 - -		3 5 - -		- 2 - -			1.1 4.1 - -	- 10 (100%) - -	5 (100%) - - -	- - - -

[a] carotid body tumour: according to Shamblin; Jugular body and tympanic body tumour: according to Fisch.

[b] imaging studies not available.

NED: no evidence of disease; *AWD*: alive with disease; *DOD*: dead of disease; *DID*: dead due to intercurrent disease.

Sixty-four of 98 patients who had their DNA tested (65%) carried a pathogenic variant in *SDHD*, of whom 50 of 64 (78%) had a positive family history for PGL or PHEO. The p.Asp92Tyr mutation in the *SDHD* gene (one of the Dutch founder mutations) was the most prevalent mutation, identified in 50% of *SDHD* mutation carriers (32/64). Three of 147 HNPGL patients (2%) developed metastatic disease, defined by the occurrence of metastatic chromaffin tissue in locoregional lymph nodes or in non-chromaffin organs distant from the primary PGL. All these three patients carried a pathogenic variant in *SDHD*. Two of three patients with metastatic disease had a concurrent PHEO. Clinical characteristics, treatment strategies and outcome of HNPGL patients with metastatic disease are outlined in Table 2.2. Treatment strategies and outcome for patients with a solitary HNPGL are outlined in Table 2.3. As different treatment strategies may apply different tumour locations within one patient, a single treatment strategy could not be associated with a patient with multiple HNPGLs.

Management

Since 1956, an increasing number of HNPGLs have been diagnosed (figure 2.1).

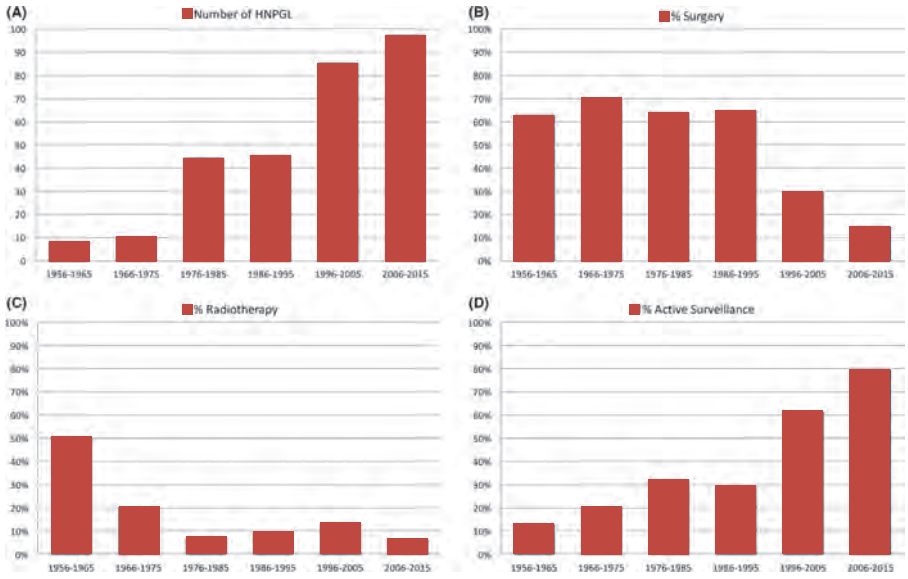

Figure 2.1 Management of head and neck paragangliomas. A, Number of diagnosed head and neck paragangliomas (HNPGLs). B, Percentage of HNPGLs that was surgically resected. C, Percentage of HNPGLs that was treated with radiotherapy. D, Percentage of HNPGL followed an active surveillance policy

Whereas the majority (64%) of HNPGLs were surgically resected in the period 1956-1995, in the last two decades surgery has been performed in a decreasing percentage of tumours (21%). Surgery was relatively frequently performed on solitary carotid body tumours (41%) and tympanic tumours (67%), whereas PGLs along the vagal nerve or at the jugular foramen were treated surgically in only 22% and 31%, respectively.
In the period 1956-1965, up to 50% of HNPGLs were treated with radiotherapy. This percentage has decreased (9% in 1966-2015) and has remained stable in the last decades. An increasing number of patients are observed (active surveillance), especially since the year 2000, coinciding with the increasing insight into the genetic determinants of PGL syndrome.

2.4 DISCUSSION

This single-centre study describes clinical characteristics and outcome of treatment in a population HNPGL patients. In accordance with earlier reports, the vast majority of HNPGLs is located at the bifurcation of the carotid artery (59%), the jugular foramen (43%), along the vagal nerve (34%) or along the tympanic nerve (22%)[2]. Importantly, 75/98 (77%) HNPGL patients who had their DNA tested were found to have a hereditary form of PGL. The majority of germline mutations in this single-centre study are found in *SDHD* (65%), comparable with previous reports on HNPGL cohorts in the Netherlands[5].

Multifocal HNPGLs were found in 54% of the patients. Multifocality was especially prevalent in *SDHD*-linked HNPGL patients (88%). This may have important ramifications for treatment decisions in this patient subgroup, even in apparently solitary tumours. As multifocal and bilateral tumours may occur synchronous or metachronous, bilateral cranial nerve involvement resulting in significant impairment of speech, swallowing and breathing has to be anticipated. If cranial nerve deficit occurs, it is usually better tolerated if the onset is slowly progressive, due to tumour progression, as opposed to a sudden paralysis due to surgery. In our series, 3/147 patients (2%) developed metastatic disease. Interestingly, these three patients were *SDHD* mutation carriers (3/64, 4.7%). This percentage for *SDHD* mutation carriers is in accordance with a previously published meta-analysis[8]. None of the 10 *SHDB* mutation carriers proved to have metastatic disease or developed a PHEO or sPGL, an observation that is most likely due to the limited number of *SDHB*-linked patients is in this cohort.

The last decades a rapidly expanding number of HNPGLs has been diagnosed in our centre. This increase is probably the result of intensified screening protocols and the introduction of DNA testing of HNPGL patients and cascade screening resulting in an early diagnosis of HNPGL in family members at risk. The management of HNPGL patients is topic of debate and has evolved considerably during the last decades. There is no universal best treatment option rather the optimal strategy is determined by a dedicated multidisciplinary team based on patient characteristics (such as age, condition and preferences), tumour characteristics (such as localisation, size, multifocality and associated cranial nerve deficits). Whether or not a patient has a germline pathogenic variant has become increasingly important in the clinical decision-making, as it has become more and more clear that the genetic predisposition is a key factor in the clinical risk profile (phenotype) of HNPGL patient subgroups. Important characteristics such as the risk of multifocality, associated sPGL en PHEO, risk of metastatic disease and even mortality seem to be highly associated with the causative gene[7].

A surgical approach is still the treatment option of choice in the majority of carotid body and tympanic tumours, tumours that can generally be surgically resected with limited surgical risk. Growing insight into the usually indolent natural course of HNPGL has resulted in a more conservative approach of tumours in which surgery would infer considerable risk to cranial nerves, that is, vagal and jugular PGL (Figure 2.1). This approach has been supported by several cohort studies, describing stable or slowly progressive tumours in a large proportion of HNPGL patients (42%-79%)[9,10]. In the Netherlands, therapeutic options (ie surgical resection, radiotherapy or surveillance) are multidisciplinary discussed, weighing potential risks and benefits of each treatment strategy per tumour and per patient.

Moving forward, more research is necessary to accurately predict the clinical behaviour of specific HNPGL tumours of individual patients, allowing for even more tailor-made management strategies, not only with regard to the natural course of the disease, but also with regard to the short- and long-term effects of possible interventions. As tumour eradication is not always possible or necessary, quality of life should be the dominant outcome parameter.

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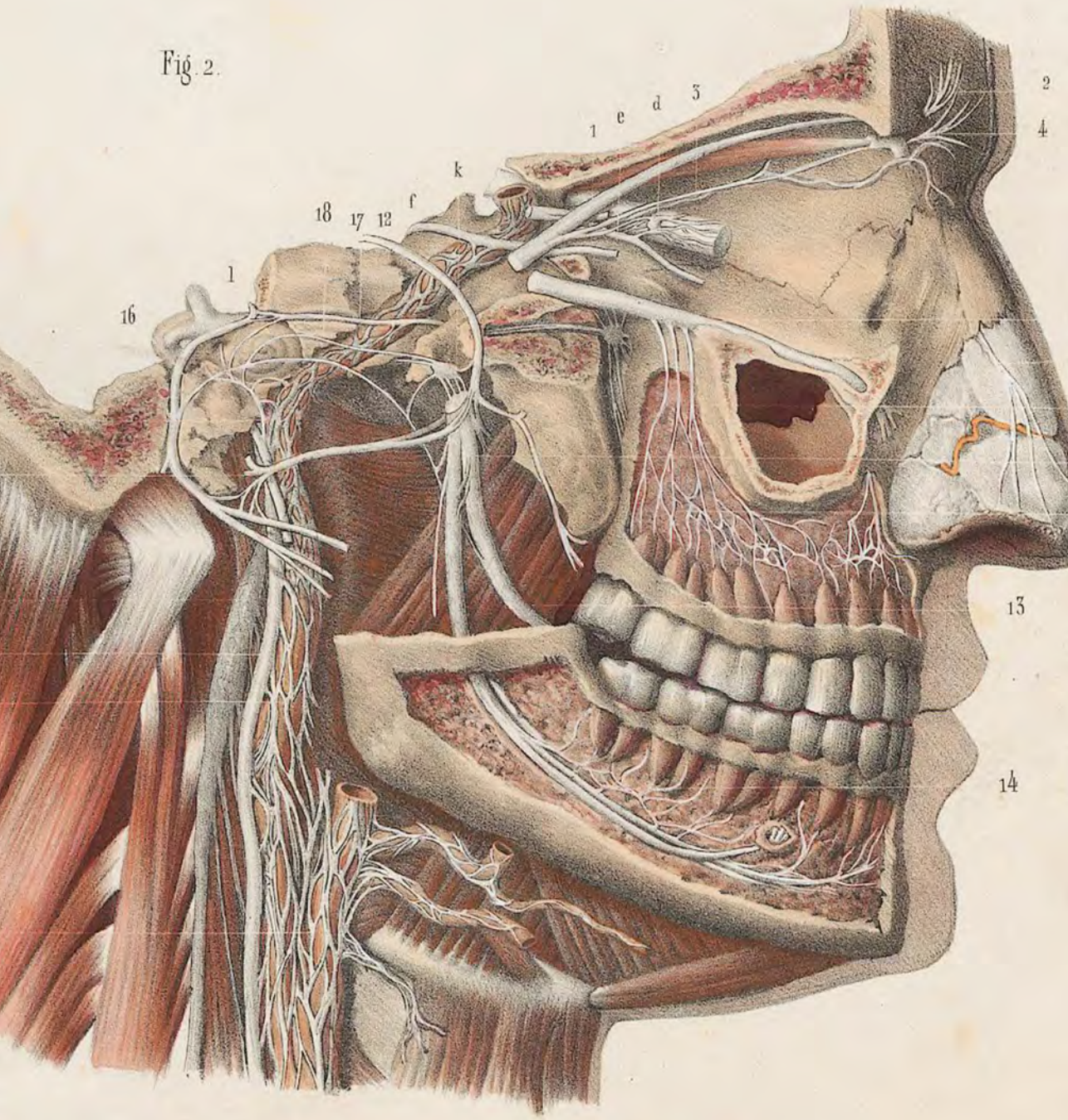
Fig. 2.



Dessinné d'après nature par Roussin
préparation par Ludovic.

3 A novel succinate dehydrogenase subunit B germline variant associated with head and neck paraganglioma in a Dutch kindred: A family-based study

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3.1 ABSTRACT

Objective: In the Netherlands, the majority of hereditary head and neck paragangliomas (HNPGL) are caused by germline variants in the succinate dehydrogenase genes (*SDHD*, *SDHB*, *SDHAF2*). Here, we evaluate a four-generation family linked to a novel *SDHB* gene variant with the manifestation of a HNPGL.

Design: A family-based study.

Setting: The VU University Medical Center (VUmc) Amsterdam, a tertiary clinic for Otolaryngology and Head and Neck Surgery.

Participants and main outcome measures: The index patients presented with an embryonic rhabdomyosarcoma and a non-Hodgkin lymphoma. Array-based comparative genomic hybridisation (aCGH) analysis and multiplex ligation-dependent probe amplification (MLPA) revealed a novel deletion of exon 1-3 in the *SDHB* gene, suspected to predispose to paraganglioma (PGL)/pheochromocytoma (PHEO) syndrome type 4. Subsequently, genetic counselling and DNA testing were offered to all family members at risk. Individuals that tested positive for this novel *SDHB* gene variant were counselled and additional clinical evaluation was offered for the identification of HNPGL and/or PHEO.

Results: The DNA of 18 family members was tested, resulting in the identification of 10 carriers of the exon 1-3 deletion in the *SDHB* gene. One carrier was diagnosed with a carotid body PGL and serum catecholamine excess, which was surgically excised. Negative *SDHB* immunostaining of the carotid body tumour confirmed that it was caused by the *SDHB* variant. The remaining 9 carriers showed no evidence of PGL/PHEO.

Conclusion: Deletion of exon 1-3 in the *SDHB* gene is a novel germline variant associated with the formation of hereditary HNPGL.

3.2 INTRODUCTION

Paragangliomas (PGLs) are rare, highly vascularised, usually benign neoplasms of paraganglia, neuroendocrine organs derived from neural crest chromaffin cells. PGLs can be found throughout the body in association with the parasympathetic or sympathetic nervous system. PGLs of the head and neck region are associated with the parasympathetic nervous system. PGL of the head and neck region are associated with the parasympathetic nervous system. They secrete catecholamines in 4%-30% of the cases, which may cause elevated blood pressure, palpitations, flushes and agitation, and may ultimately result in severe cardiovascular complications[1-3]. Head and neck paragangliomas (HNPGLs) are most commonly found at the carotid bifurcation (60%), but can also arise at the jugular bulb, along the vagal nerve or the tympanic nerve[4]. The closely related pheochromocytoma (PHEO), also known as adrenal PGL, together with the thoracic and abdominal extra-adrenal PGL are paraganglion tumours associated with the sympathetic nervous system.

In about 40% of the patients with an apparently sporadic presentation of PGL or PHEO, a genetic predisposition can be identified[4]. There is considerable genetic heterogeneity in PGL and PHEO, currently over 30 different genes have been associated with PGL/PHEO formation. The majority of HNPGL, extra-adrenal PGL and PHEO are caused by germline variants in genes encoding subunits and cofactors of the succinate dehydrogenase (SDH) enzyme complex (A, B, C, D, AF2) [5-7]. The associated syndromes are quite distinct. *SDHB*-linked tumour syndrome is usually characterised by single tumours, and gene variant carriers develop more frequently extra-adrenal PGLs, PHEOs and metastatic disease than carriers in the other subunits of the *SDH* gene[8]. Furthermore, *SDHB* gene variants are implicated in the development of renal cell carcinoma, papillary thyroid carcinoma and GIST tumours[8-10].

In this study, we describe the occurrence of HNPGL in a four-generation family linked to a novel *SDHB* gene variant. The index patients are 2 young sisters that did not present themselves with a HNPGL or PHEO, but with a rhabdomyosarcoma and a non-Hodgkin lymphoma. Array-based comparative genomic hybridization (aCGH) analysis and subsequent multiplex ligation-dependent probe amplification (MLPA) revealed a deletion of exon 1-3 in the *SDHB* gene in both patients that has not been described previously. Although *SDHB* immunostaining of the rhabdomyosarcoma and non-Hodgkin lymphoma showed that there was no causal relationship between the *SDHB* gene variant and these tumours, this novel exon deletion was suspected to predispose to PGL/PHEO syndrome type 4 based on the

characteristics of the gene variant itself. Subsequently, genetic counselling and DNA testing were offered to all family members. Clinical evaluation of the germline variant carriers revealed an asymptomatic carotid body PGL in an aunt of the index patients.

3.3 MATERIALS AND METHODS

All the participating family members gave written informed consent for the clinical study and DNA test. In case of individuals under 18 years of age, written informed consent was obtained from their parents.

Data were collected from the VU University Medical Center (VUmc), Amsterdam, the Netherlands, a tertiary referral centre for HNPGL and/or PHEO in the Netherlands. Family members at risk were offered genetic counselling and pre-symptomatic screening as part of the protocol for standard care of pathogenic *SDHB* variant carriers at risk in the Netherlands[11]. *SDHB* gene variant analysis was performed by the Leiden Genome Technology Center (LGTC) of the Leiden University Medical Center (LUMC, Leiden, the Netherlands), using Sanger sequencing on an ABI 377 (Applied Biosystems, Carlsbad, CA) Genetic Analyzer and multiplex ligation-dependent probe amplification (MLPA), P266 MLPA-kit (MRC Holland, Amsterdam, the Netherlands).

Germline variant carriers were offered annual clinical surveillance for PGL and PHEO at the departments of Otolaryngology/Head and Neck Surgery and Endocrinology/Metabolic diseases of the VUmc. Annual biochemical screening for excessive catecholamine excretion included the measurement of (nor)adrenaline, vanillylmandelic acid (VMA), dopamine, (nor)metanephrine and/or 3-methoxythyramine (3-MT) in two 24-hour urinary samples, and/or plasma-free (nor)metanephrine and/or 3-methoxythyramine (3-MT). All carriers were offered magnetic resonance imaging (MRI) of the thorax/abdomen/pelvis once every 2 years and head and neck region once every 3 years. Upon detection of a HNPGL (a carotid body tumour) and catecholamine excess in one carrier, a multidisciplinary team consisting of otolaryngologists, endocrinologists, geneticists, radiotherapists and vascular surgeons advised surgical resection of the PGL. The surgery was performed at the VUmc, Amsterdam, the Netherlands. *SDHB* immunostaining was performed on tumour tissue according to the protocol described elsewhere[12].

3.4 RESULTS

The index patients, 2 sisters (13 and 16 years old), were referred to the Department of Clinical Genetics of the VU University Medical Center for etiologic evaluation because of a medical history of a embryonic rhabdomyosarcoma and a non-Hodgkin lymphoma, respectively. Array-based comparative genomic hybridisation (aCGH) and multiplex ligation-dependent probe amplification (MLPA) in both sisters revealed a 16p12.2 microdeletion, a 20q12 deletion, and a novel exon 1-3 deletion in the *SDHB* gene.

Based on positive *SDHB* immunostaining of the two index tumours, no causal relationship could be established between the *SDHB* gene variant and the occurrence of these tumours. Even so, on grounds of the characteristics of the *SDHB* gene variant alone, it was suspected that this variant could be pathogenic. Both a deletion in *SDHB* exon 1 and in exon 3 are known to cause hereditary PGL syndrome type 4 and predispose to HNPGL, PHEO, extra-adrenal PGL and malignant PGL/PHEO[13-15]. Despite the lack of apparent symptomatic neuroendocrine tumours in this family, cascade screening was offered to the family members of these 2 patients.

Subsequently, 18 relatives at risk belonging to a four-generation family with a total of 43 members were tested for this specific *SDHB* exon deletion. Eight individuals tested negative and were considered not to be at risk of PGL/PHEO formation. Ten family members (6 women, 4 men) were carriers of the deletion of exon 1-3 in the *SDHB* gene (figure 3.1).

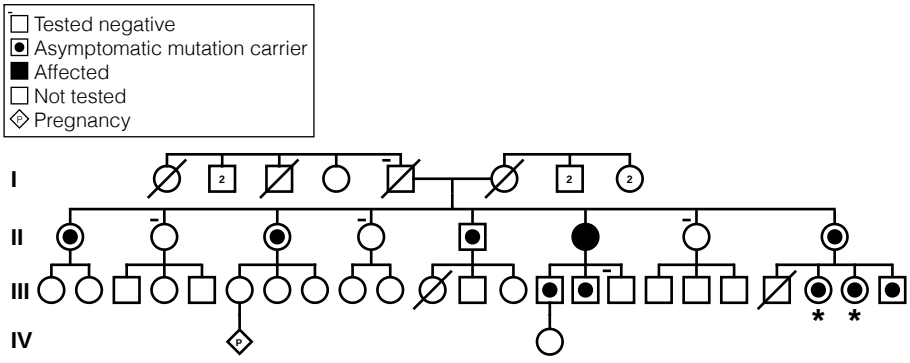

Figure 3.1 Pedigree of the SDHB-linked family. The asterisks show the index patients.

The mean age at detection of the gene variant was 34 years (range 11-57). The mean duration of follow-up was 26 months (range 12.6-32.3). None of the germline variant carriers presented with signs or symptoms suggestive of a PGL/PHEO during clinical examination. However, a slight increase in plasma-free (nor)metanephrine or 3-MT levels was observed in 5 variant carriers (50%) at the time of diagnosis (table 3.1). In one of these patients, pre- and post-contrast enhanced 3D time-of-flight (TOF) MR angiography of the head and neck region revealed a mass at the right carotid bifurcation, suggestive of a carotid body PGL (figure 3.2).

Increased plasma-free normetanephrine levels were observed at time of diagnosis and 24-hour ambulatory blood pressure monitoring revealed blood pressure peaks. MR imaging of the thorax, abdomen and pelvis showed no other localisation of a PGL or PHEO. Additional iodine-123-meta-iodobenzylguanidine (MIBG) scintigraphy did not unequivocally identify the source of the catecholamine overproduction. A multi-

Table 3.1 Phenotype of 10 relatives carrying the exon 1-3 deletion in *SDHB*.

	Sex	Age at diagnosis	PGL location	Catecholamine biochemistry at diagnosis	Additional mutation	Other tumor (in history)
1	F	39	None	Normal (serum)	16p12.2 del	
2	M	29	None	Normal (serum)	None	
3	M	23	None	Elevated NM (serum) Elevated M (serum) Elevated 3-MT (serum)	None	
4	F	56	None	Elevated NM (serum) Elevated M (serum) Elevated 3-MT (serum)	None	
5	F	53	None	Elevated NM (serum) Normal M (serum) Normal 3-MT (serum)	None	
6	M	51	None	Elevated NM (serum) Elevated M (serum) Normal 3-MT (serum)	None	
7	F	46	Carotid body PGL†	Elevated NM (serum) Normal M (serum) Normal 3-MT (serum)	None	
8	F	16	None	Normal (urine)	None	Non-Hodgkin Lymphoma‡
9	F	13	None	Normal (urine)	16p12.2 del 20q12 del	Embryonal rhabdomyosarcoma‡
10	M	10	None	Normal (urine)	16p12.2 del	

F, female; *M*, male; *PGL*, paraganglioma; *NM*, normetanephrine; *3-MT*, 3-methoxythyramine; †, *SDHB*-associated; ‡, not-associated.

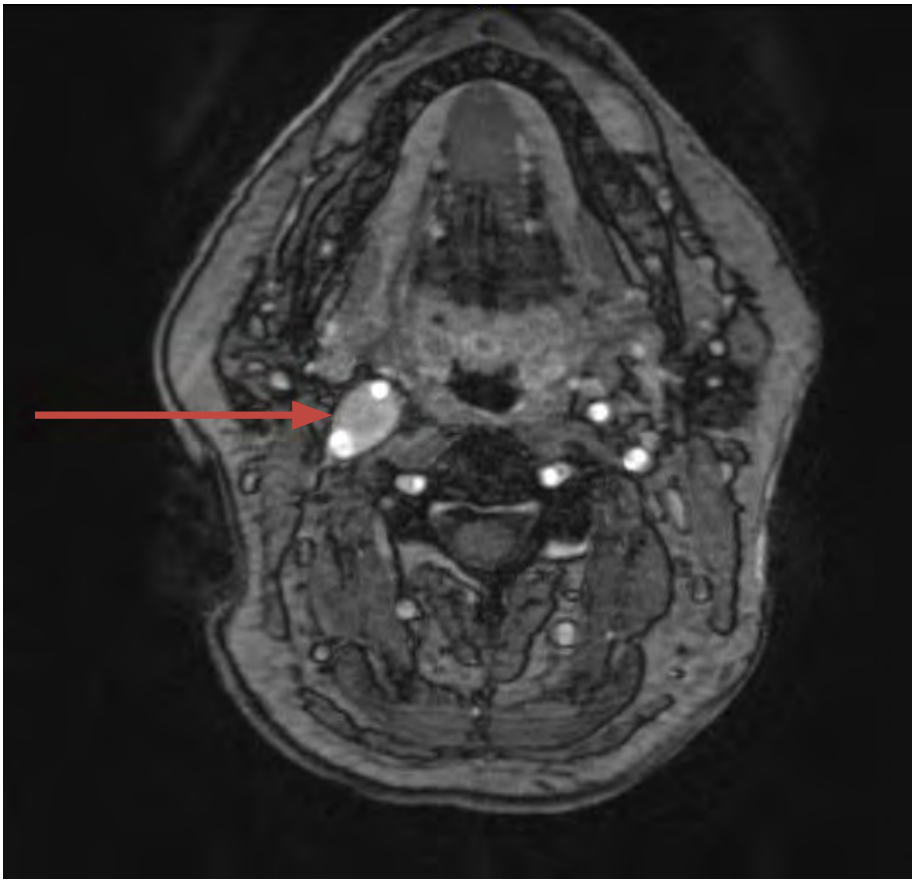

Figure 3.2 Axial magnetic resonance imaging (MRI) of the head and neck region showing a Shamblin type II carotid body paraganglioma. 3D TOF (time-of-flight angiography) sequence is used to visualise flow within vessels, without the need to administer contrast intravenously.

disciplinary team consisting of otolaryngologists, endocrinologists, radiotherapists and vascular surgeons advised surgical resection on grounds of the causative *SDHB* gene variant and catecholamine excess. An alpha blockade protocol until the day of surgery was followed, because of increased preoperative plasma levels of catecholamine and observed peaks in blood pressure. The carotid body PGL was removed in total via a transcervical approach in an uncomplicated procedure (figure 3.3).
Histologic evaluation confirmed the diagnosis HNPGL, and negative *SDHB* immunostaining indicated the causal relation between the *SDHB* exon 1-3 deletion and the carotid body PGL. Biochemical evaluation 4 months after surgery showed normalisation of plasma-free normetanephrine levels.

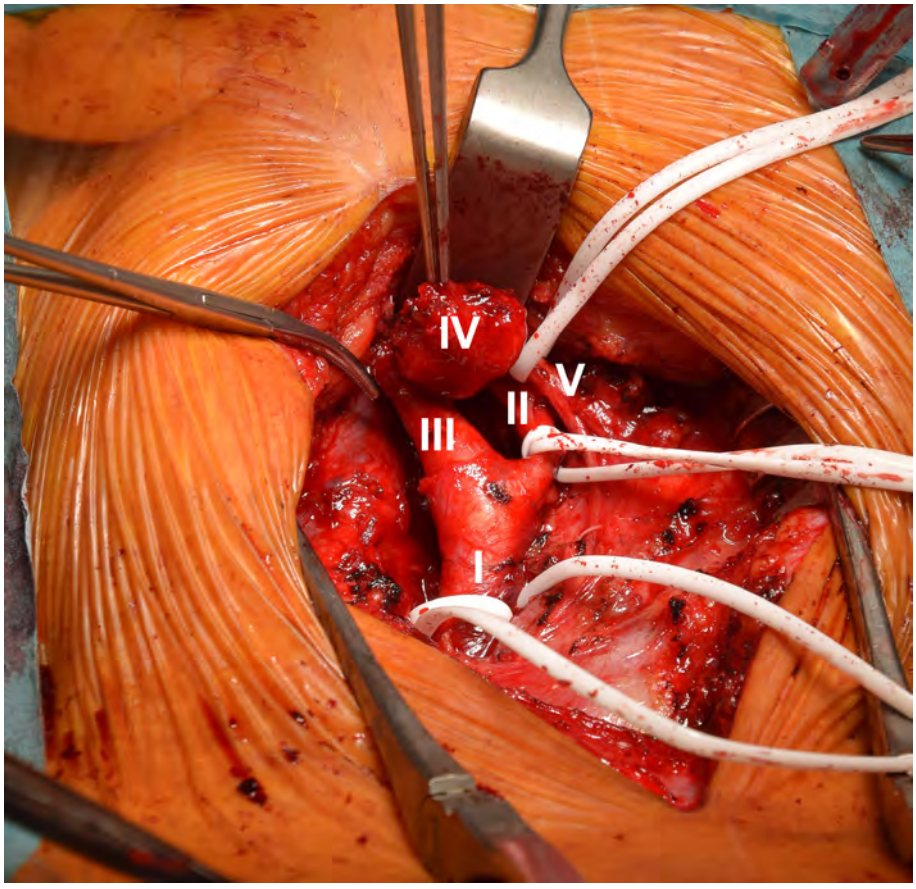

Figure 3.3 Surgical resection, via a transcervical approach, of the paraganglioma located between the right internal and external carotid arteries, and its close relationship with the hypoglossal nerve. I, common carotid artery; II, external carotid artery; III, internal carotid artery; IV, paraganglioma; V, hypoglossal nerve.

3.5 DISCUSSION

In this report, we describe a novel deletion of exon 1-3 in *SDHB* causing HNPGL formation. We tested 18 relatives belonging to a four-generation family consisting of 43 members, of whom 10 were identified to carry this novel *SDHB* germline variant. One patient was diagnosed with an asymptomatic carotid body PGL and serum catecholamine excess. Negative *SDHB* immunostaining of the tumour tissue confirms the association between this novel *SDHB* variant and the carotid body PGL[4].

Interestingly, the two paediatric index patients were diagnosed with a non-Hodgkin lymphoma and an embryonal rhabdomyosarcoma. It is now known that *SDHx* germline variants do not only predispose to PGL/PHEO, but also to non-paraganglionic tumours such as gastrointestinal stromal tumours, renal cell carcinomas and pituitary adenomas[8-10]. The complete spectrum of the *SDHB*-linked phenotype has, however, not yet been fully elucidated, because the *SDHx* genes are not routinely evaluated in individuals with non-endocrine tumours. A recent study has highlighted a possible role for *SDHx* gene variants in lymphoid malignancies. One *SDHB* variant carrier has been described with a Hodgkin lymphoma and an abdominal extra-adrenal PGL, and one *SDHC* variant carrier with Hodgkin lymphoma and a positive family history of PHEO/GIST[16]. However, both the Hodgkin lymphoma and normal lymphoid tissues of the *SDHB* variant carrier displayed minimal *SDHB* staining, precluding definitive assessment of *SDHB* protein loss. Based on positive *SDHB* immunostaining of rhabdomyosarcoma and lymphoma tissue in our index patients, a causal relationship between the deletion of exon 1-3 in *SDHB* and the occurrence of these tumours could not be established.

Only 1 patient was affected with an apparently asymptomatic carotid body PGL, detected at the age of 47. The risk of developing a PGL or PHEO (or penetrance) in *SDHB* germline variant carriers is subject of recent debate. Initially, it was estimated to range between 50% and 70% at 50 years of age[7,9,17]. These estimates are probably inflated, as recent studies using more thorough pedigree analysis and a more robust statistical correction for the ascertainment bias show a lower age-dependent penetrance of *SDHB* variants, approximately 9%-21% at 50 years. These reports also indicate that there are no significant differences in the penetrance of different types of *SDHB* variants. The low penetrance of the *SDHB* variant found in this family is in line with these recent reports[18-20].

The management of HNPGL is challenging and requires a tailor-made approach. The management strategy is based on several important factors, such as patient characteristics (ie age, comorbidities and patient preferences) and tumour characteristics (ie localisation, size, growth rate, biochemical activity and multicentricity). The causal gene variant plays an increasingly prominent role in the clinical decision-making, as different genes confer different risks and are associated with different clinical phenotypes. *SDHB* variants causing PGL syndrome type 4 are usually associated with single PGL/PHEO that have a higher risk of progression to metastatic disease than PGL/PHEO associated with other *SDH* genes[7,17]. This study identifies a novel deletion of exon 1-3 in *SDHB* causing HNPGL. Cascade testing of family members at risk identified a patient with a pre-symptomatic carotid body tumour, elevated catecholamine levels and high blood pressure, which normalised after surgical resection of the tumour.

3.6 CONCLUSION

In this report, we present a novel deletion of exon 1-3 in the *SDHB* gene associated with the formation of hereditary HNPGL. The penetrance of this gene variant seems low. Cascade screening of family members carrying this mutation is important to detect pre-symptomatic PGL. Especially in case of catecholamine-producing tumours, timely intervention may prevent cardiovascular complications.

3.7 REFERENCES

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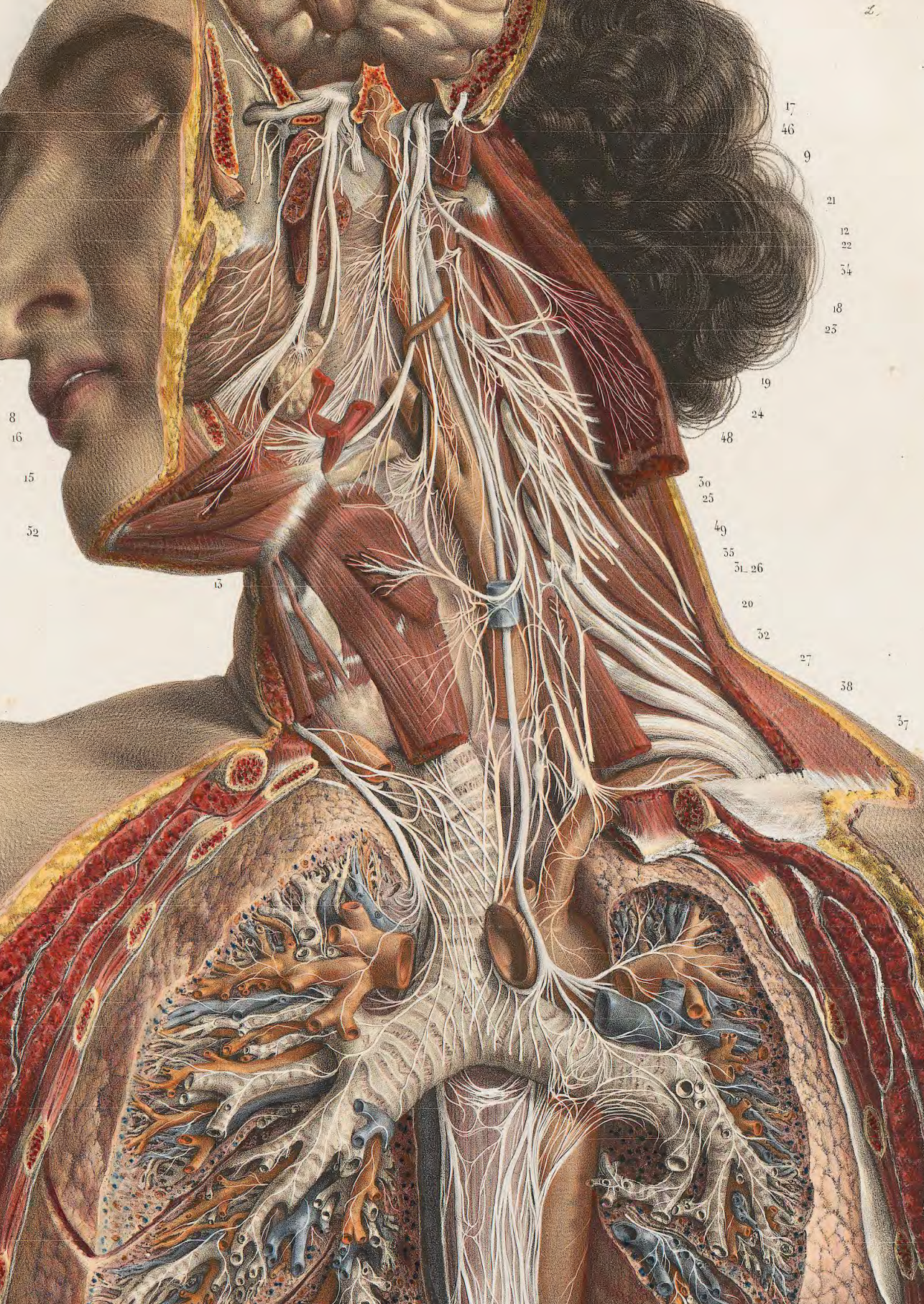
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4 Low penetrance of paraganglioma and pheochromocytoma in an extended kindred with a germline *SDHB* exon 3 deletion

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4.1 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.

4.2 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)[5,8,9-17]. 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.

4.3 MATERIALS AND METHODS

Data were collected from 2 tertiary referral centres 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.

4.4 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.

Subsequently, the mutation status of 49 of his relatives belonging to a four-generation family with 153 members was evaluated (figure 4.1). 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).

Six mutation carriers (35%) were diagnosed with PGL (table 4.1). 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 of 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 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 (figure 4.2).

Table 4.1 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

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

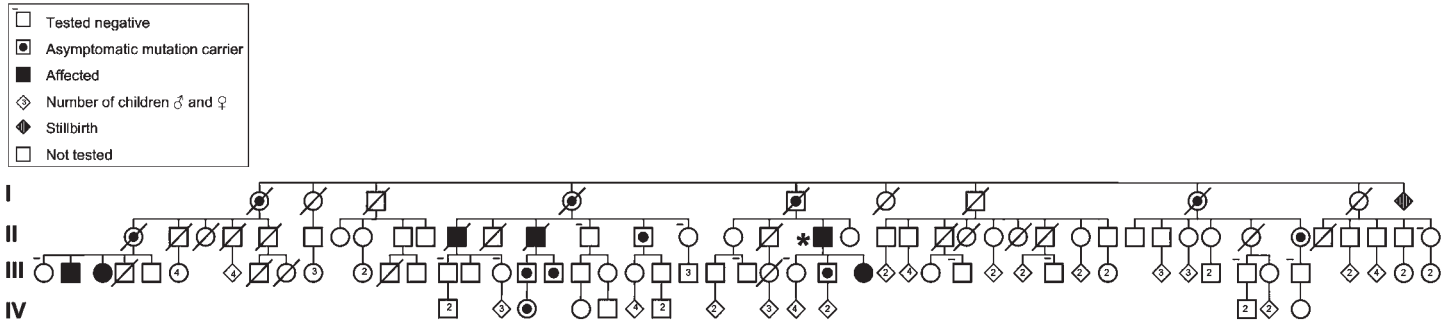

Figure 4.1 The pedigree of the *SDHB*-linked family. The asterisk shows the index patient.

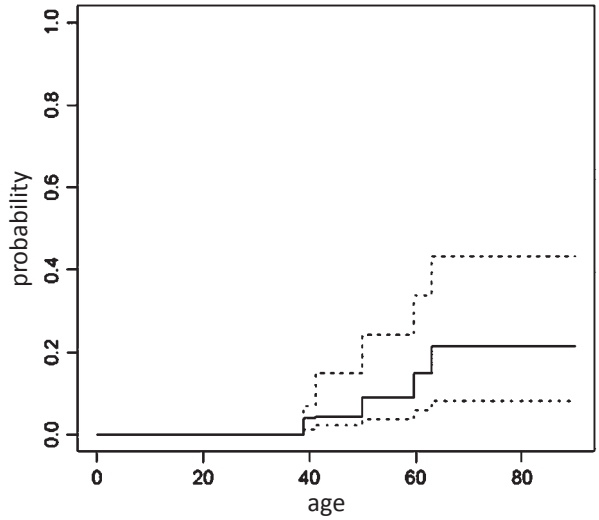

Figure 4.2 Estimated age-related penetrance of the *SDHB* exon 3 deletion in the family presented. Solid line: maximum likelihood estimated of the age-related penetrance. Upper dashed line: estimated upper bound of the age-related penetrance (Kaplan-Meier curve assuming all non-tested family members 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).

4.5 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 paraganglioma 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 amongst 41 mutations 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 age 50 years)[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, since the pedigree presented in this study is large and since 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 Figure 4.2).

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.

4.6 ACKNOWLEDGEMENTS

We thank all members of the family for participating in this study.

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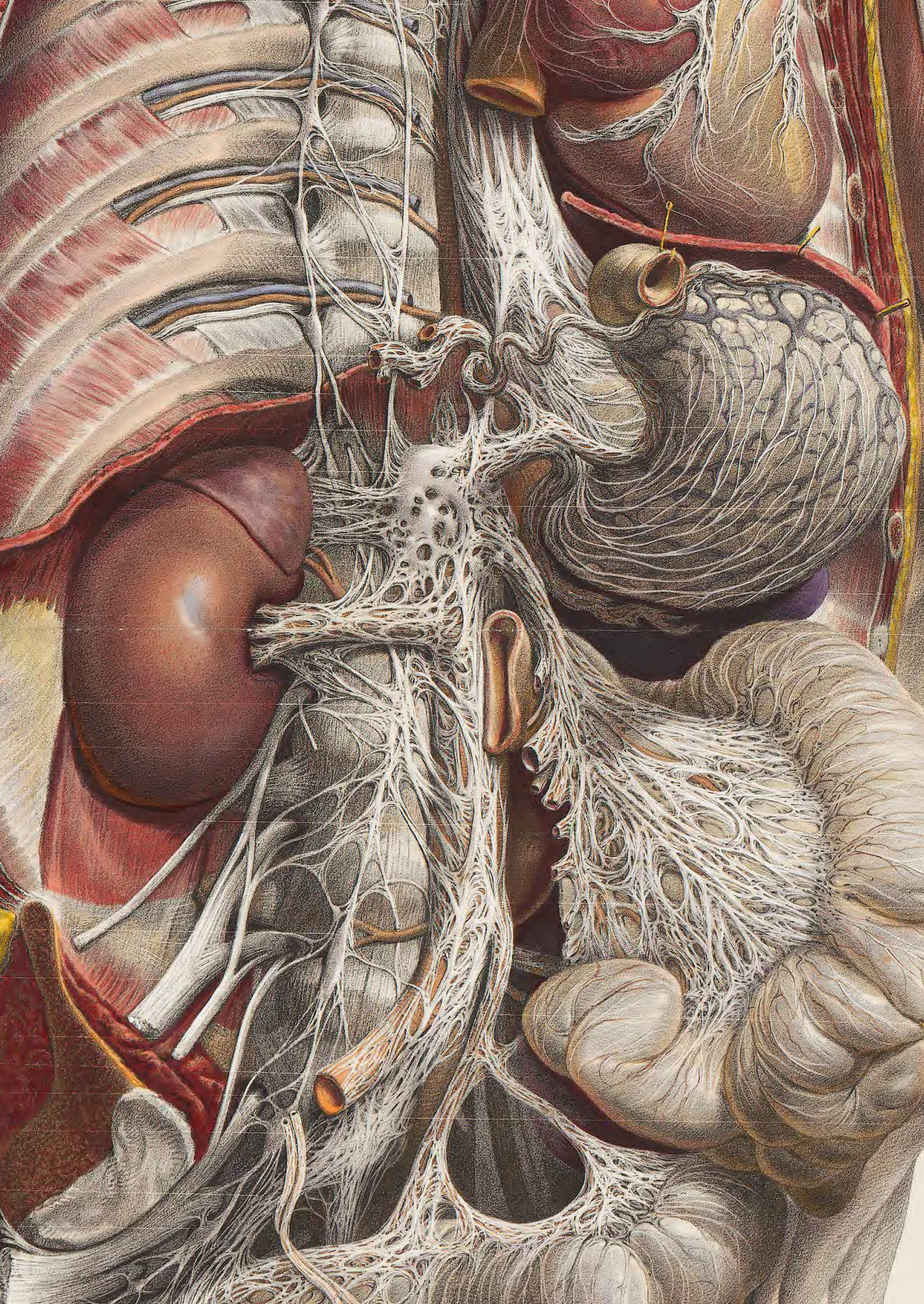

5 The phenotype of *SDHB* germline mutation carriers: a nationwide study

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5.1 ABSTRACT

Objective: Succinate dehydrogenase B subunit (*SDHB*) gene germline mutations predispose to pheochromocytomas, sympathetic paragangliomas, head and neck paragangliomas and non-paraganglionic 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* germline mutation carriers and assess differences in clinical phenotypes related to specific *SDHB* mutations.

Design: Retrospective descriptive study.

Methods: Retrospective descriptive study in seven academic centers.

Results: We included 194 *SDHB* mutation carriers consisting 65 (33.5%) index patients and 129 (66.5%) relatives. Mean age was 44.8 ± 16.0 years. Median duration of follow-up was 2.6 years (range: 0–36). Sixty persons (30.9%) carried the exon 3 deletion and 46 (23.7%) the c.423 + 1G > A mutation. Fifty-four mutation carriers (27.8%) had one or multiple head and neck paragangliomas, 4 (2.1%) had a pheochromocytoma and 26 (13.4%) had one or more sympathetic paragangliomas. Fifteen patients (7.7%) developed metastatic paraganglioma and 17 (8.8%) developed non-paraganglionic tumors. At study close, there were 111 (57.2%) 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 metastatic disease or other tumors between carriers of the two founder *SDHB* mutations (exon 3 deletion vs c.423 + 1G > A).

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

5.2 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 the Netherlands, succinate dehydrogenase (*SDH*) germline 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[2]. The *SDHAF2* gene encodes SDH complex assembly factor 2 (*SDHAF2*), essential for flavination of the SDHA protein and SDH enzyme activity[3]. 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[4]. Furthermore, *SDHB* mutation carriers more frequently develop sPGLs, PCCs and metastatic disease than mutation carriers in the other subunits of the *SDH* gene[5-7]. Although initial malignancy rates as high as 31–97% were reported for *SDHB*-related PGL[5-9], we recently reported risks of metastatic 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 metastatic 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[10].

SDH mutations have also been linked to non-paraganglionic 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[11].

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[12,13]. 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* germline mutation carriers in a nationwide study in seven academic centers in the Netherlands.

5.3 SUBJECTS AND METHODS

In this retrospective nationwide study, all *SDHB* germline 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 on July 1, 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 identified in seven of the eight clinical genetics centers of 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). Maastricht University Medical Center was not able to participate for technical reasons. However, they only had identified one germline *SDHB* mutation carrier. Data from 47 *SDHB* mutation carriers from the Leiden University Medical Center are previously described by van Hulsteijn *et al.*[14].

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 an 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 germline *SDHB* mutations is performed by direct sequencing using the Sanger method on an ABI 377 Genetic Analyser (Applied Biosystems) and by multiplex ligation-dependent probe amplification (MLPA) using the P226 MLPA kit (MRC Holland, Amsterdam, the Netherlands). *SDHB* germline variants are classified as in the international guidelines by Plon *et al.*[15]. In this manuscript we report pathogenic or likely pathogenic variants, including missense mutations in highly conserved regions that are likely pathogenic, as germline mutations.

All *SDHB* germline 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, abdomen and pelvis 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 (PET) 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. In cases without available tumor histology, tumors were classified as paraganglionic based on their specific characteristics on CT and/or MRI. When in doubt, additional nuclear medicine imaging studies were performed in order to confirm the diagnosis.

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

An unaffected mutation carrier was defined as a germline mutation carrier without evidence of disease (i.e. HNPGL, sPGL and/or PCC). A disease-affected mutation carrier was defined as a germline 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) 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. For

comparison of disease risks for index patients and relatives Kaplan–Meier curves (One Minus Cum Survival) were plotted. Results are presented as mean ± s.d. Differences were considered statistically significant at $P \leq 0.05$ (two-sided).

5.4 RESULTS

A total of 194 *SDHB* germline 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), 17 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, 83 men (42.8%) and 111 women (57.2%) were included. The median duration of the follow-up was 2.6 years (range: 0–36). Eleven persons (5.7%) were lost to follow-up: six 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.

In total, our cohort consisted of 83 (42.3%) disease-affected mutation carriers and 111 (57.2%) unaffected mutation carriers. From the 111 unaffected mutation carriers, 104 have had complete radiological screening (CT/MRI of the head and neck region and CT/MRI of the thorax/abdomen/pelvis). Seven have had either a CT/MRI of the head and neck region (two mutation carriers) or a CT/MRI of the thorax/abdomen/pelvis (five mutation carriers). From the 83 disease-affected mutation carriers, 74 have had complete radiological screening. Nine mutation carriers have had either a CT/MRI of the head and neck region (two mutation carriers) or a CT/MRI of the thorax/ abdomen/pelvis (seven mutation carriers). However, all the mutation carriers, who did not have had complete radiological screening by CT/MRI, did had another (total body) imaging study (i.e. ^{123}I MIBG-scans/^{18}F-FDG PET-scans/^{18}F-DOPA PET-scans).

There were 65 index patients and 129 relatives of index patients. Of the 129 relatives, 109 persons (84.5%) 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 renal

cell carcinomas (RCCs) and a gastric gastrointestinal stromal tumor (GIST), one was thought to have an 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).

Genetics

Details of *SDHB* mutations are outlined in table 5.1.

Sixty (30.9%) were carriers of the exon 3 deletion and 46 (23.7%) were carriers of the c.423 + 1G > A mutation. The c.654G > A, p.(Trp218*) mutation was present in 19 persons (9.8%) and the c.653 G > C, p.(Trp218Ser) mutation in 11 persons (5.7%).

Table 5.1 *SDHB* germline mutations.

DNA mutation	SDHB predicted protein change	Number of subjects (%)
exon 3 deletion	p.?	60 (31)
c.423+1G>A	p.?	46 (24)
c.654G>A	p.(Trp218*)	19 (10)
c.653G>C	p.(Trp218Ser)	11 (6)
c.574T>C	p.(Cys192Arg)	8 (4)
c.200+1G>A	p.?	6 (3)
c.137G>A	p.(Arg46Gln)	4 (2)
c.328A>C	p.(Thr110Pro)	4 (2)
c.418G>T	p.(Val140Phe)	4 (2)
c.725G>A	p.(Arg242His)	3 (1.5)
c.649C>T	p.(Arg217Cys)	3 (1.5)
c.590C>G	p.(Pro197Arg)	3 (1.5)
c.686_725del	p.(Glu229fs)	3 (1.5)
c.343C>T	p.(Arg115*)	3 (1.5)
c.292T>C	p.(Cys98Arg)	2 (1)
deletion promoter and exon 1	p.?	1 (0.5)
deletion promoter till exon 8	p.0	2 (1)
exon 2 deletion	p.?	2 (1)
exon 1 deletion	p.?	2 (1)
c.713delT	p.(Phe238fs)	1 (0.5)
c.727T>A	p.(Cys243Ser)	1 (0.5)
c.761C>T	p.(Pro254Leu)	1 (0.5)
c.626C>T	p.(Pro209Leu)	1 (0.5)
c.380T>C	p.(Ile127Thr)	1 (0.5)
c.325A>C	p.(Asn109His)	1 (0.5)
c.1A>G	p.?	1 (0.5)
c.119A>C	p.(Lys40Thr)	1 (0.5)

Clinical features

The mean age at first evaluation at the outpatient clinic was 44.8 ± 16.0 years (range 11–76). In total, our cohort comprised of 65 (33.5%) index patients and 129 (66.5%) 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 5.2.

Table 5.2 Clinical phenotypes of specific *SDHB* germline mutations.

	Total cohort (n = 194)	Exon 3 deletion (n = 60)	c.423+1G>A (n = 46)	c.654G>A (n = 19)	c.653G>C (n = 11)
Gender					
Man	83 (42.8%)	29 (48.3%)	18 (39.1%)	8 (42.1%)	2 (18.2%)
Woman	111 (57.2%)	31 (51.7%)	28 (60.9%)	11 (57.9%)	9 (81.8%)
Age (mean±SD)[a]	44.8 ± 16.0	43.2 ± 15.3	51.0 ±14.5	44.0 ± 18.1	49.1 ± 11.7
Family history positive	129 (66.5%)	40 (66.7%)	35 (76.1%)	18 (94.7%)	8 (72.7%)
HNPGL	54 (27.8%)	18 (30.0%)	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 (11.3%)	6 (10.0%)	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 (6.2%)	4 (6.6%)	3 (6.5%)	0	1 (9.1%)
Left	6	2	0		1
Right	6	2	3		0
Bilateral	0	0	0		0
JBT	14 (7.2%)	7 (11.7%)	5 (10.9%)	0	0
Left	8	5	3		
Right	5	1	2		
Bilateral	1	1	0		
Tymp	10 (5.2%)	4 (6.7%)	1 (2.2%)	0	0
Left	5	1	1		
Right	5	3	0		
Bilateral	0	0	0		
Other (HNPGL)	1 (right tonsil)	0	0	0	0
Age HNPGL[b]	45.9 ± 14.2	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 (2.1%)	1 (1.7%)	0	0	1 (9.1%)
Left	3	1			1
Right	1	0			0
sPGL[c]	26 (13.4%)	8 (13.3%)	5 (10.9%)	1 (5.3%)	1 (9.1%)
Operation sPGL	25	8 (100%)	5 (100%)		1 (100%)
Malignant PGL/PCC	15 (7.7%)	5 (8.3%)	1 (2.2%)	1 (5.3%)	1 (9.1%)

Table 5.2 Continued

	Total cohort (n = 194)	Exon 3 deletion (n = 60)	c.423+1G>A (n = 46)	c.654G>A (n = 19)	c.653G>C (n = 11)
Other tumors[d]	17 (8.8%)	5 (8.3%)	7 (15.2%)[g]	0	0
Mamma ca.	1	0	1		
Renal cell ca.	3[e]	2	1		
Basal cell ca.	2	0	1		
Melanoma	2	1	1		
Lung ca.	1	0	1		
Prostate ca.	1	0	0		
Colon ca.	2	0	2		
Meibomian gland	1	0	0		
Synovial sarcoma	1	1	0		
Ovarian ca.	1	0	1		
Gastric GIST	2[f]	0	1		
Micro-PRL	1	0	0		
Pituitary incidentaloma	1	1	0		
Disease status at last follow-up					
NED	133 (68.6%)	42 (70.0%)	32 (69.6%)	16 (84.2%)	8 (72.7%)
AWD	43 (22.2%)	13 (21.7%)	9 (19.6%)	1 (5.3%)	3 (27.3%)
LTF	11 (5.7%)	3 (5.0%)	2 (4.3%)	1 (5.3%)	0
DOD	4 (2.1%)	2 (3.3%)	1 (2.2%)	1 (5.3%)	0
DID	3 (1.5%)	0	2 (4.3%)	0	0

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

[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.

Table 5.3 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)

F, female; *M*, male; *MN*, metanephrine; *NA*, not assessed; *NAV*, not available; *NED*, no evidence of disease; *NMN*, normetanephrine

[a] Age at diagnosis of pheochromocytoma.

Table 5.4 Characteristics of 26 patients with sympathetic paragangliomas.

Case	Sex	*SDHB* mutation	Location sPGL	Age[a] (y)	Malignant disease	Tumor reduction therapy	Outcome
1	F	c.343C>T	Retroperitoneal and pre-sacral	31	No	Surgery	No evidence of disease
2	F	Exon 3 deletion	Para-aortic	41	No	Surgery (non-radical)	Alive with disease
3	M	c.200+1G>A	Retroperitoneal (pararenal)	42	Yes	Surgery, ^{131}I-MIBG therapy, radiotherapy	Alive at age 52, with disease.
4	M	Exon 3 deletion	Retropancreatic	11	No	Surgery	No evidence of disease
5	M	Exon 3 deletion	Thoracic (vertebra Th6) and intra-abdominal	10 and 32	Yes	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	Surgery	No evidence of disease
7	M	Exon 3 deletion	Para-aortic abdominal	42	No	Surgery	No evidence of disease
8	F	c.423+1G>A	Retroperitoneal	36	No	Surgery	No evidence of disease
9	F	c.725G>A	Para-adrenal	40	Yes	Surgery, Lutetium octreotate therapy	Alive at age 51, with disease
10	F	c.423+1G>A	Para-iliac (2 lesions)	19	No	Surgery	No evidence of disease
11	M	c.423+1G>A	Para-aortic abdominal	31	No	Surgery	No evidence of disease
12	F	c.653G>C	Retroperitoneal	66	Yes	Surgery, ^{131}I-MIBG therapy	Alive at age 78, with disease
13	M	Exon 3 deletion	Retroperitoneal	37	Yes	Surgery	Alive at age 40, with disease
14	M	Exon 3 deletion	Bladder and retroperitoneal	27	No	Surgery	No evidence of disease
15	M	c.423+1G>A	Para-aortic abdominal	38	No	Surgery	No evidence of disease
16	M	c.325A>C	Para-aortic abdominal	30	Yes	Surgery, ^{131}I-MIBG therapy	Alive at age 46, with disease
17	M	c.200+1G>A	Bladder	45	Yes	Surgery, radiotherapy, chemotherapy (CVD)	Alive at age 47, with disease
18	M	c.574T>C	Liver hilum	24	No	Surgery	No evidence of disease
19	F	c.727T>A	Retroperitoneal (para-aortic)	52	Yes	Surgery, radiotherapy	Died at age 63, due to intercurrent disease
20	F	c.343C>T	Thoracic	14	No	Surgery	Loss to follow-up
21	F	c.686_725del	Para-aortic abdominal and para-vertebral (Th3/Th4)	39	No	Follow-up	Alive with disease

Table 5.4 Continued

Case	Sex	*SDHB* mutation	Location sPGL	Age[a] (y)	Malignant disease	Tumor reduction therapy	Outcome
22	M	c.626C>T	Bladder	42	Yes	Radiotherapy, Firstmappp trial (started June 2014)	Alive at age 52, with disease
23	F	c.423+1G>A	Para-renal	31	No	Surgery	No evidence of disease
24	M	Exon 3 deletion	Presacral	28	Yes	Surgery, ^{131}I-MIBG therapy, radiotherapy	Dead of disease: died at age 32 due to progressive disease
25	F	c.654G>A	Bladder	19	Yes	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	Yes	Surgery, ^{131}I-MIBG therapy, radiotherapy	Dead of disease: died at age 37 due to progressive disease

CVD, cyclophosphamide, vincristine, dacarbazine; *F*, female; 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; *M*, male; *PGL*, paraganglioma; *RFA*, radiofrequency ablation;
[a]Age at diagnosis of sympathetic paraganglioma;

Of the whole cohort, 54 mutation carriers (27.8%) were clinically affected with one or multiple HNPGLs. Mean age of diagnosis of HNPGL was 45.9 ± 14.2 years (range: 11–77). Carotid body tumors were the most prevalent HNPGLs (in 11.3%), followed by jugular body tumors (in 7.2%) and vagal body tumors (in 6.2%). Twenty-seven carriers (50.0%) had an operation for their HNPGL and 15 (27.8%) received radiotherapy.

Four patients (2.1%) 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 5.3.

Twenty-six mutation carriers (13.4%) were clinically affected with one or more sPGLs. Mean age of diagnosis of sPGL was 33.4 ± 12.7 years (range: 10–66). None of the 26 mutation carriers suffered from an HNPGL. More than half of the patients with an sPGL had elevated hormone levels. 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 metastatic disease and three of them died due to progressive metastatic disease. Clinical characteristics and biochemical phenotypes are detailed in table 5.4.

Out of the whole cohort of *SDHB* germline mutation carriers, 15/194 (7.7%) developed metastatic PGL. Clinical characteristics, treatment and outcome of the patients with metastatic disease are displayed in detail in table 5.5.
Treatment of the primary tumor existed of surgery in all patients. None of the 47 mutation carriers described previously have developed metastatic disease since our publication in 2014[14].
Seventeen mutation carriers (8.8%) developed a total of 21 non-paraganglionic tumors. Three patients developed a total of five (histology confirmed) renal tumors. Four of those tumors were described previously and classified as *SDH*-deficient renal carcinomas[11,16,17]. Two patients developed a RCC on one side (one clear cell carcinoma and one *SDH*-deficient carcinoma), and one patient developed two foci of a RCC on the left side and one on the right side (all three *SDH*-deficient renal carcinomas). This latter patient also developed an *SDH*-deficient gastric GIST and has been described previously[11]. There was one other patient with an *SDH*-deficient 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).

Table 5.5 Clinical characteristics of patients with metastatic paragangliomas.

Case	Sex	*SDHB* mutation	Location PGL	Age[a] (y)	Age[b] (y)	Location metastases	Treatment malignant disease	Outcome
1	M	c.200+1G>A	Retroperitoneal (pararenal)	42	45	Bone	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, 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, radiotherapy	LTF, follow-up till age 22, alive with disease.
4	F	c.725G>A	Para-adrenal	40	45	Lymph nodes, bone	Surgery and ^{177}Lutetium octreotate therapy	Alive at age 51, with disease
5	M	c.423+1G>A	Jugular body	48	57	Bone (vertebra)	None	Died at age 57, due to rapidly progressive malignant disease
6	F	Exon 3 deletion	Carotid body	35	66	Lymph nodes, bone	None (not within study period)	Alive at age 66, with disease
7	F	c.653G>C	Retroperitoneal	66	70	Lymph nodes, bone	^{131}I-MIBG therapy	Alive at age 78, with disease
8	M	Exon 3 deletion	Retroperitoneal	37	38	Lymph nodes	Surgery	Alive at age 40, with disease
9	M	c.325A>C	Para-aortic abdominal	30	39	Lymph nodes, bone, lung	^{131}I-MIBG therapy	Alive at age 46, with disease
10	M	c.200+1G>A	Bladder	45	45	Lymph nodes, bone, lung	Surgery, radiotherapy, chemotherapy (CVD)	Alive at age 47, with disease
11	F	c.727T>A	Retroperitoneal (para-aortic)	52	55	Bone	Radiotherapy	Died at age 63, due to intercurrent disease
12	M	c.626C>T	Bladder	42	46	Lymph nodes, bone	Radiotherapy, Firstmappp trial (started June 2014)	Alive at age 52, with disease
13	M	Exon 3 deletion	Presacral	28	28	Bone	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	sunitinib	Died at age 62 due to progressive disease
15	F	Exon 3 deletion	Para-vertebral abdominal	33	33	Lymph nodes, bone	Surgery, ^{131}I-MIBG therapy, radiotherapy	Died at age 37 due to progressive disease

CVD, cyclophosphamide, vincristine, dacarbazine; *F*, female; 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; *LTF*, loss to follow-up; *M*, male; *PGL*, paraganglioma; *RFA*, radiofrequency ablation; *Th6*, 6th thoracic vertebra.

[a] age at diagnosis of paraganglioma;

[b] age at diagnosis of malignant disease.

Besides these malignancies, one person developed a microprolactinoma and one person had a non-functioning pituitary incidentaloma, both of which underwent radiological follow-up without available biopsy or surgically-resected material. Of these 17 mutation carriers with non-paraganglionic tumors, only three patients had also paraganglionic tumors (all three patients had an HNPGL). The clinical characteristics of the index patients vs relatives are outlined in table 5.6 and the age-related disease risk for index patients (probands) vs relatives is outlined in figure 5.1.

Table 5.6 Clinical characteristics of index patients and relatives.

	Age (mean ✣ SD)	Follow-up (median, year)	HNPGL (%)	PCC (%)	sPGL (%)	Malignant PGL/PCC
Index patients (65)	43.6 ± 14.8	4.5	38 (58.5)	3 (4.6)	21 (32.3)	15 (23.1)
Relatives (129)	45.4 ± 16.6	2.0	16 (12.4)	1 (0.8)	5 (3.9)	0

HNPGL, head and neck paraganglioma; *PCC*, pheochromocytoma; *sPGL*, sympathetic paraganglioma.

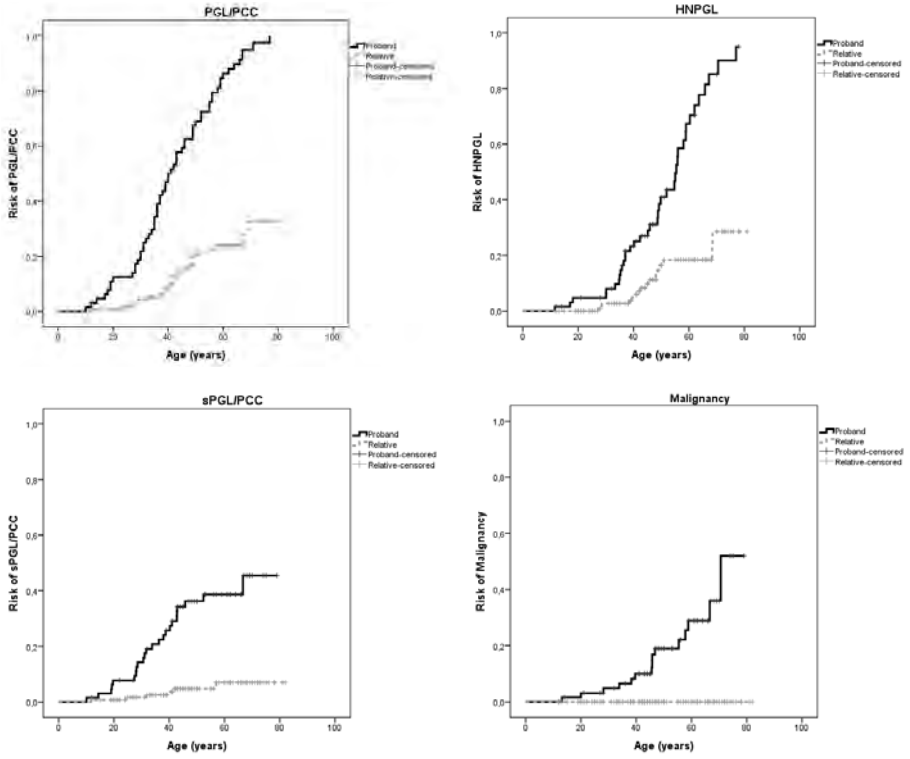

Figure 5.1 Comparison of age at onset in *SDHB* mutations carriers: index patients (probands) vs relatives. PGL/PCC, risk of (all) paragangliomas/pheochromocytoma; HNPGL, risk of head and neck paraganglioma; sPGL/PCC, risk of sympathetic paraganglioma/pheochromocytoma; malignancy, risk of malignancy.

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.

5.5 DISCUSSION

In this nationwide multicenter study we assessed the phenotypes of 194 *SDHB* germline mutation carriers. Our cohort consisted of 83 (42.8%) disease-affected mutation carriers and 111 (57.2%) unaffected mutation carriers. Fifty-four carriers (27.8%) were clinically affected with one or multiple HNPGLs. Only four patients (2.1%) were clinically affected with a PCC and 26 (13.4%) with one or more sPGLs. Fifteen patients (7.7%) developed metastatic disease.

Previous studies have reported much higher rates for developing PCC and sPGLs, 18–52% and 59–84% respectively[5,6,8,18]. 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 (19). 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 (2.1% and 13.4% 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 (111 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 (27.8%) among *SDHB* mutation carriers compared with other studies (3–31%)[5,6,8,18] and even when compared with that of the French network (14.5%)[19]. 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[5,6,8]. 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 an 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 metastatic PGL. In three of these patients (20%) the primary tumor was an HNPGL (including one in the tonsil) and in 12 patients (80%) the primary tumor was an sPGL. Removal of all unaffected mutation carriers (111 subjects) results in a prevalence of metastatic disease by 18.1% (15/83) in PGL/PCC patients. Taking into account only the sPGL patients, the malignancy risk is as high a 46.2% (12/26). For HNPGL patients, this malignancy rate was 5.6% (3/54). This means that the malignancy risk for patients already suffering from an sPGL is high, which has implications for the follow-up of those patients. Srirangalingam *et al.* reported metastatic PGL in five of 16 (31%) disease-affected subjects[8]. 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 to 97%[5-9]. 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[7,8]. 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 metastatic PGL of 13 and 23% respectively[10], also much lower than previously reported[20,21]. In the fifteen patients with metastatic 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 to 17 years[7] and Srirangalingam *et al*. between 1.5 and 25 years[8]. Because it is not possible to diagnose malignancy based on histopathology of the primary tumor, only if metastatic disease is present, the current and previously reported wide

ranges of time to metastatic transformation underscore the need for an extended follow-up in patients with an *SDHB* mutation, especially in disease-affected mutation carriers. The median duration of follow-up is 2.6 years in this study, which is a limitation of this study. However, the follow-up time is relatively short due to a shorter follow-up of relatives compared to index patients. Future studies with a longer duration of follow-up are needed to validate our results.

Our findings show a relatively mild phenotype of *SDHB* mutations in the Netherlands. One might hypothesize that this could be associated with the low altitude and therefore relatively high oxygen levels in the Netherlands[22]. However, studying a large cohort from a single country provides 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[18]. 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[23]. 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 metastatic disease and a relatively high number of HNPGLs compared with previous reports of referral-based cohorts. This is most probably not a regional phenomenon but the result of the more comprehensive inclusion of unaffected mutation carriers, underlining the importance of including both disease-affected and unaffected individuals in studies that assess the phenotype of germline mutations. It furthermore highlights the importance of thorough tumor screening protocols that include radiology of the head and neck region in *SDHB* mutation carriers.

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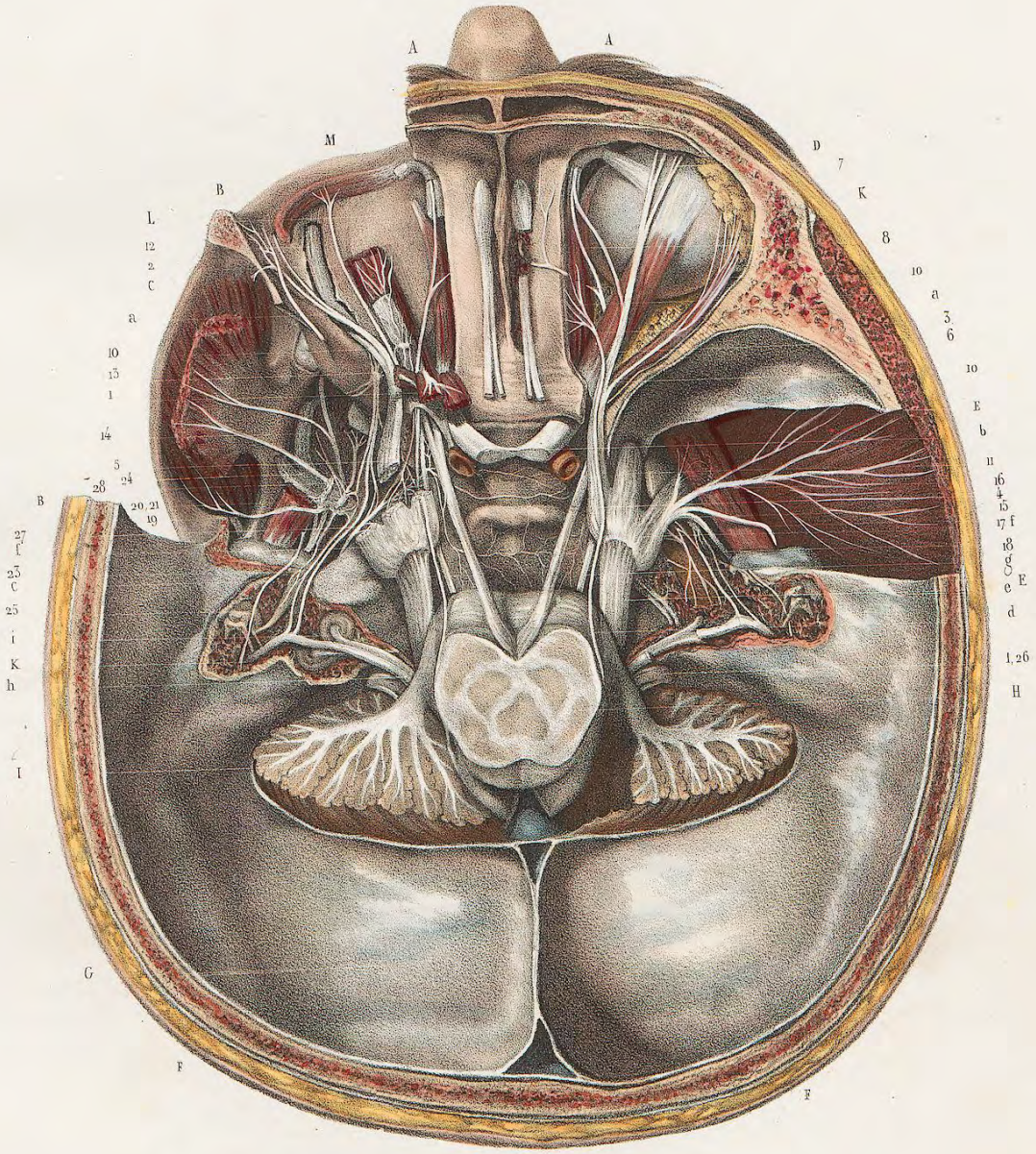

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6 Nationwide study of head and neck paraganglioma patients carrying *SDHB* germline mutations

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6.1 ABSTRACT

Background: Germline mutations in the succinate dehydrogenase B (*SDHB*) gene predispose to hereditary paraganglioma (PGL) syndrome type 4. The aim of this study was to evaluate the clinical characteristics and outcome of treatment strategies for patients with head and neck paraganglioma (HNPGL) carrying *SDHB* germline mutations.

Method: This was a retrospective evaluation of patients with HNPGL carrying *SDHB* germline mutations in the Netherlands.

Results: In a Dutch nationwide cohort study of *SDHB* germline mutation carriers, 54 patients with a total of 62 HNPGLs were identified. Forty-one of 54 patients (76 per cent) visited the outpatient clinic because of associated complaints. Eight patients (15 per cent) had multiple PGLs. One patient (2 per cent) developed a phaeochromocytoma and three (6 per cent) developed a malignant PGL. Twenty-seven patients (50 per cent) had an operation for their HNPGL and 15 (28 per cent) received radiotherapy. Three patients with HNPGL (6 per cent) were diagnosed with additional non-paraganglionic tumours.

Conclusion: If an *SDHB* germline mutation is identified in a patient with HNPGL, the clinician should be aware of the variable manifestations of the *SDHB*-linked tumour syndrome, the risk of catecholamine excess, concurrent phaeochromocytoma, and association with non-paraganglionic tumours.

6.2 INTRODUCTION

Paragangliomas (PGLs) of the head and neck are predominantly benign hypervascular tumours that arise from neural crest cells of the autonomic nervous system. Head and neck paragangliomas (HNPGLs) most frequently originate from the paraganglia in the bifurcation of the carotid artery, the jugular foramen, along the vagus nerve or along the tympanic nerve[1]. Other locations are the nasal cavity, paranasal sinuses, parotid gland, cervical sympathetic chain, pharynx, larynx, trachea, aortic arch, ciliary ganglion and thyroid gland[2]. HNPGLs are associated with extra-adrenal PGLs arising in the thorax and abdomen, predominantly along the sympathetic trunk, and with phaeochromocytomas of the adrenal gland. These extra-adrenal PGLs and phaeochromocytomas usually present with signs and symptoms of catecholamine excess[3]. Generally HNPGLs are parasympathetic in origin, and symptoms depend on the localization, tumour size, compression of surrounding structures and associated cranial nerve deficits. Between 4 and 30 per cent of HNPGLs secrete catecholamines[4,5]. HNPGLs can occur spontaneously or as part of a hereditary syndrome. A rapidly expanding number of genes are associated with hereditary PGL. Hereditary PGL syndrome is caused most frequently by genes encoding succinate dehydrogenase (*SDH*) subunits or co-factors (*SDHA/B/C/D/AF2* genes). Other associated genes are *RET*, *NF1*, *VHL*, *HIF2A*, *FH*, *TMEM127* and MAX[6,7]. In the Netherlands, mutations in *SDHD, SDHB* and *SDHAF2* are responsible for most hereditary cases. *SDHD*-related PGLs are usually characterized by multiple PGLs located predominantly in the head and neck region, with a low frequency of malignancy. In contrast, *SDHB* mutation carriers are reported to develop single PGLs and metastatic PGLs more frequently[8-12]. Recently it has become clear that the *SDHB*-linked tumour syndrome not only comprises PGLs and phaeochromocytomas, but also non-paraganglionic tumours such as renal clear cell carcinoma, gastrointestinal stromal tumours (GISTs) and pituitary tumours[6-12].

In a recently published nationwide evaluation of 194 *SDHB* mutation carriers[13], 54 patients (27.8 per cent) were identified with *SDHB*-linked HNPGLs. In the present study, the clinical characteristics and clinical course, treatment modalities and outcome of these patients with HNPGL linked to *SDHB* mutations were evaluated.

6.3 METHODS

Patients with HNPGL were identified in a Dutch nationwide cohort of *SDHB* germline mutation carriers. The genotype and phenotype of this nationwide cohort have been described elsewhere[13]. *SDHB* mutation carriers and patients with PGL were investigated in multiple centres according to structured protocols used for standard care of PGL in the Netherlands[14,15]. Carriers were offered annual clinical surveillance for concurrent HNPGL, concurrent phaeochromocytomas and extra-adrenal PGLs in departments of otorhinolaryngology and endocrinology. For *SDHB* mutation carriers over 18 years of age, surveillance consisted of MRI of the head and neck region once every 3 years, and MRI or CT of the thorax, abdomen and pelvis once every 2–3 years. At the time of this study there were no national structured protocols for surveillance of *SDHB* mutation carriers aged less than 18 years. Therefore, the method and interval of surveillance in this age category varied between centres.

When HNPGL was diagnosed, treatment or intensified periodic examination was offered, guided by tumour characteristics such as location, size (defined as the largest diameter of the HNPGL on imaging), growth rate, associated symptoms, and patient characteristics such as age, general condition and co-morbidity, according to local protocols. A wait and scan policy, radiotherapy, surgical resection, or combinations thereof, were possible treatment strategies. Annual biochemical screening included the measurement of adrenaline (epinephrine), noradrenaline (norepinephrine), vanillylmandelic acid (VMA), dopamine (D), metanephrine, normetanephrine and/or 3-methoxytyramine (3-MT) in two 24-h urinary samples, and/or plasma free (nor)metanephrine and/or 3-MT. In case of excessive catecholamine secretion (any value above the upper reference limit), radiological assessment by MRI or CT of the thorax, abdomen and pelvis and/or [^{123}I]metaiodobenzylguanidine (MIBG)scan/PET with 2-deoxy-2-[fluorine-18]fluoro-D-glucose (^{18}F-FDG PET)/^{18}F-L-dihydroxyphenylalanine (^{18}F-DOPA) PET was performed to identify potential sources of excessive catecholamine production outside the head and neck region. As no histological features of the primary tumour reliably distinguish benign from malignant (HN)PGLs, malignant disease was defined as the presence of metastases (paraganglionic cells in non-neuroendocrine tissue distant from the primary tumour).

After obtained informed consent, clinical, radiological and genetic data of patients with HNPGL were collected. Duration of the follow-up was defined as the time from the date of first presentation to the most recent outpatient visit within the study interval.

The study was approved by the medical ethics committee of Leiden University Medical Centre (number P13.161); participating centres complied with their local medical ethics committee requirements. SPSS® version 20.0 (IBM, Armonk, New York,USA) was used for data analysis.

6.4 RESULTS

Clinical status

In all, 54 patients, 28 female (52 per cent) and 26 male (48 per cent), with a total of 62 HNPGLs were identified in a nationwide evaluation of *SDHB* mutation carriers[13]. The mean age of diagnosis was 45·9 (range 11–77) years. Sixteen patients (30 per cent) had a positive family history, and 38 (70 per cent) presented with a negative family history (table 6.1). The mean duration of follow-up was 7·8 (median 4·5, range 0·1–36·9) years.

Table 6.1 Clinical characteristics of patients with *SDHB*-linked head and neck paragangliomas.

	Negative family history (*n* = 38)	Positive family history (*n* = 16)
Age at diagnosis (years)*	47 (12–77)	44 (28–83)
Sex ratio (M : F)	19 : 19	7 : 9
Malignant paraganglioma	3	0
Multiple head and neck paragangliomas	6	1
Phaeochromocytoma	1	0
Extra-adrenal paraganglioma	0	0
Carotid body tumour	11	11
Jugular body tumour	13	1
Vagal body tumour	8	4
Tympanic body tumour	9	1

*Values are mean (range).

Genetics

In all, 21 different *SDHB* germline mutations were identified (table 6.2). The most prevalent *SDHB* germline mutations are known as Dutch founder mutations – a deletion of exon 3 (18 patients, 33 per cent) and the c.423+1G>A mutation (11 patients, 20 per cent).

Table 6.2 Details of *SDHB* germline mutations in patients with a head and neck paraganglioma.

cDNA mutation	Protein alteration	No. of patients
Exon 3 deletion*	p.?	18
c.423+1G>A*	p.?	11
c.653G>C	p.(Trp218Ser)	3
c.137G>A	p.(Arg46Gln)	3
c.200+1G>A	p.?	2
c.328A>C	p.(Thr110Pro)	2
c.686_725del	p.(Glu229fs)	1
c.725G>A	p.(Arg242His)	1
c.761C>T	p.(pro254Leu)	1
Exon 1 deletion	p.?	1
Promoter to exon 8 deletion	p.0	1
Promoter and exon 1 deletion	p.?	1
c.119A>C	p.(Lys40Thr)	1
c.649C>T	p.(Arg217Cys)	1
c.1A>G	p.?	1
c.590C>G	p.(Pro197Arg)	1
c.292T>C	p.(Cys98Arg)	1
c.654G>A	p.(Trp218*)	1
c.380T>C	p.(Ile127Thr)	1
c.418G>T	p.(Val140Phe)	1
c.574T>C	p.(Cys192Arg)	1

* Dutch founder mutations.

Presenting symptoms

Thirteen patients (24 per cent) had no associated signs or symptoms at the time of diagnosis, and the tumour was identified as a result of presymptomatic screening of known *SDHB* mutation carriers (11 patients) or as an incidentaloma (2). Forty-one patients (76 per cent) with HNPGL came to medical attention as a result of HNPGL-associated signs or symptoms. The occurrence and type of presenting symptoms depended on the location of the tumour in the head and neck region (figure 6.1).

The majority of patients with tympanic and jugulotympanic PGLs presented with symptoms or signs (20 of 24, 83 per cent), mostly hearing loss and pulsatile tinnitus, whereas the majority with a vagal body PGL (8 of 12, 67 per cent) had no symptoms at the time of diagnosis. Cranial nerve deficit (causing hoarseness, dysphagia and hypoglossal palsy) was most commonly seen in jugular PGLs. Seven carotid body tumours were asymptomatic and the tumour was identified coincidentally (as an incidentaloma) or through presymptomatic testing (7 of 24, 29 per cent).Nineteen *SDHB* carriers with an HNPGL presented with hypertension (19 of 54, 35 per cent).

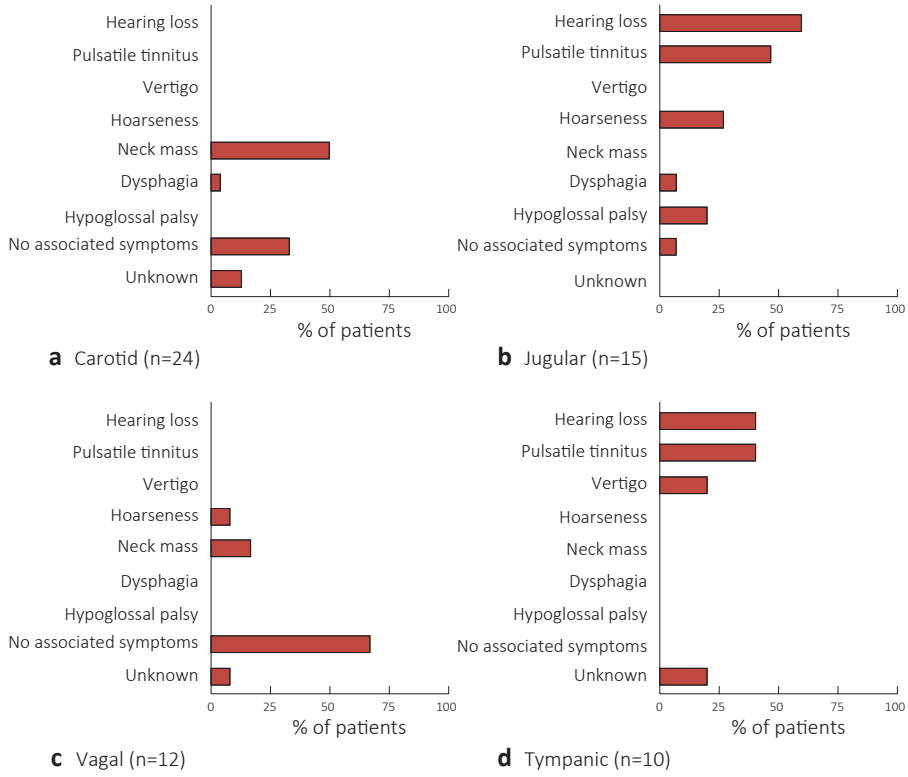

Figure 6.1 Presenting symptoms in patients with *SDHB*-linked head and neck paraganglioma (HNPGL). The proportion of patients with HNPGL who presented with a specific symptom is shown per tumour site. Some patients did not have any symptoms, and the HNPGL was identified coincidentally (as an incidentaloma) or through presymptomatic screening. The single paraganglioma that was diagnosed in the tonsil is not shown. a Carotid, b Jugular, c Vagal, d Tympanic.

Multicentricity and non-paraganglionic tumours

Multiple PGLs were present in eight (15 per cent) of the 54 patients with HNPGL to a maximum of three concurrent tumours (table 6.3). In five patients, multiple HNPGLs were discovered during initial imaging. Two patients were initially diagnosed with a solitary HNPGL and developed a second, metachronous, HNPGL during follow-up. One patient (2 per cent) underwent an adrenalectomy because of a phaeochromocytoma 36 years before the diagnosis of a vagal body PGL. No concurrent extra-adrenal PGLs were identified in this *SDHB*-linked HNPGL patient cohort. Three patients (6 per cent) were diagnosed with non-paraganglionic tumours additional to their HNPGL: a melanoma, a pituitary microprolactinoma and

Table 6.3 Clinical characteristics, treatment strategies and outcome of patients with multiple *SDHB*-linked head and neck paragangliomas.

Sex	*SDHB* mutation	Presenting symptoms	Tumour and location	Age at diagnosis (years)	Treatment			Outcome	Follow-up (years)
					Strategy	Tumour treated	Age (years)		
F	c.653G>C	Unknown	VBTL, PHEO	19	Surgery	PHEO	19	AWD	37
F	Exon 3 deletion	Hearing loss, pulsatile tinnitus, swelling neck, hoarseness	CBTR, JBTR, JBTL	30	RT	JBTL	30	AWD	2
M	c.761C>T	Swelling neck	CBTL, JBTR	33	Surgery	CBTL	33	AWD	28
M	c.423+1G>A	None (PST)	VBTR, JBTL	49	RT	JBTL	56	AWD	6
F	Exon 3 deletion	Pulsatile tinnitus	VBTR, JBTL	49	Surgery	JBTL	49	AWD	15
						VBTR	50		
					RT	JBTL	65		
M	Exon 3 deletion	Pulsatile tinnitus	VBTL, TBTR	52	RT	TBTR	52	AWD	2
F	c.649C>T	Swelling neck	CBTR, CBTL	55	Surgery	CBTL	55	AWD	14
M	c.590C>G	None (incidentaloma)	CBTR, CBTL	56	Watchful waiting			AWD	10

VBTL, vagal body tumour left; *PHEO*, phaeochromocytoma; *AWD*, alive with disease; *CBTR*, carotid body tumour right; *JBTR*, jugular body tumour right; *JBTL*, jugular body tumour left; *RT*, radiotherapy; *CBTL*, carotid body tumour left; *PST*, presymptomatic screening; *VBTR*, vagal body tumour right; *TBTR*, tympanic body tumour right.

low-grade B-cell non-Hodgkin lymphoma. Although multiple non-paraganglionic tumours have been shown to be part of the *SDHB*-linked tumour spectrum, *SDHB* immunostaining was not performed on the non-paraganglionic tumours found in this study, and so no definitive causal relation with the *SDHB* germline mutation could be established[16].

Location and size

The most frequently found paraganglioma locations within the head and neck region were the jugular foramen (25 tumours: 14 left, 11 right), the carotid bifurcation (24 tumours: 13 left, 11 right) and along the vagal nerve (12 tumours: 6 left, 6 right) (table 6.1). One patient had a PGL in the right tonsil. Of 24 patients with a jugulotympanic tumour, ten had an isolated tympanic tumour (Fisch type A or B[17]). One of the ten patients with a tympanic PGL had a concurrent carotid body HNPGL.

Mean tumour size at first presentation differed depending on the location of the tumour; the mean size on initial imaging of vagal PGL was 35 (range 4–70) mm, followed by carotid body PGL (28 (4–58) mm), jugular PGL (26 (17–44) mm) and tympanic PGL (10 (4–22) mm).

Malignancy

Three patients with HNPGL (6 per cent) developed metastatic disease (tables 6.1 and 6.4). Initially, these three patients had solitary, seemingly benign, HNPGLs. They developed metastases during follow-up at 2·2, 9·2 and 31·3 years after initial diagnosis. No clear associations between the occurrence of metastatic disease and genetic factors such as *SDHB* mutation type, or clinical factors such as age of the patient, size of the initial tumour or location of the initial tumour, were found (table 6.4).

Table 6.4 Clinical characteristics, treatment strategies and outcome of patients with malignant *SDHB*-linked head and neck paragangliomas.

Sex	*SDHB* mutation	Age (years)*	Age (years)†	Location	Size of primary tumour at initial diagnosis (mm)	Location of metastases	Catecholamine excess at diagnosis	Treatment of primary tumour	Treatment of metastases	Outcome
F	c.418G>T	18	20	Right tonsil	20	Lymph nodes, bone (vertebra)	Urinary level raised (3-MT); plasma normal	Surgery	Surgery and RT	AWD at age 22 years; subsequently lost to follow-up
M	c.423+1G>A	48	57	JBTL	Unknown	Bone (vertebra)	Urinary level raised (VMA, D, A, NA); plasma not measured	Surgery and RT (at age 57 years)	None	Died from disease at age 57 years
F	Exon 3 deletion	35	66	CBTL	48	Lymph nodes, bone	Urine negative; plasma not measured	Surgery and RT (at age 66 years) (recurrent CBTL)	None	AWD at age 66 years

*Age at diagnosis of head and neck paraganglioma; †age at diagnosis of metastatic disease. *3-MT*, 3-methoxytyramine; *RT*, radiotherapy; *AWD,* alive with disease; *JBTL,* jugular body tumour left; *VMA*, vanillylmandelic acid; *D*, dopamine; *A*, adrenaline (epinephrine); *NA*, noradrenaline (norepinephrine); *CBTL*, carotid body tumour left.

Catecholamine excess

Screening for catecholamine excess was performed at the time of diagnosis and at annual intervals during follow-up by urine and/or plasma analysis in 52 of the 54 patients. In all, 27 (52 per cent) of these 52 patients tested positive for catecholamine excess during follow-up. At the time of diagnosis, 14 patients tested positive for adrenaline (epinephrine), noradrenaline (norepinephrine) or their metabolites, and seven tested positive for dopamine or its metabolite. The results of catecholamine screening in the three patients with metastatic HNPGL is outlined in table 6.4.

Treatment strategy and outcome

Twenty-seven patients (50 per cent) had an operation and 15 (28 per cent) received radiotherapy, either as single modality or as adjuvant therapy. In 19 patients (35 per cent) no intervention was performed. Treatment strategies and outcome for patients with a solitary HNPGL are outlined in table 6.5.

Table 6.5 Overall outcome and treatment strategy in patients with a solitary *SDHB*-linked head and neck paraganglioma.

Tumour location	Overall outcome	Mean follow-up (years)	Treatment		Outcome			
			Strategy	*n*	NED	AWD	DFD	LTF
Carotid body tumour (*n* = 18)	NED 9	7.8	Watchful waiting	6	–	6	–	–
	AWD 7		Surgery	11	9	–	–	2
	DFD 0		RT	–	–	–	–	–
	LTF 2		Surgery + adjuvant RT	1	–	1	–	–
Jugular body tumour (*n* = 10)	NED 0	7.6	Watchful waiting	4	–	3	–	1
	AWD 8		Surgery	1	–	1	–	–
	DFD 1		RT	1	–	1	–	–
	LTF 1		Surgery + adjuvant RT	4	–	3	1	–
Tympanic body tumour (*n* = 9)	NED 6	8.2	Watchful waiting	2	–	2	–	–
	AWD 3		Surgery	4	4	–	–	–
	DFD 0		RT	2	–	2	–	–
	LTF 0		Surgery + adjuvant RT	1	1	–	–	–
Vagal body tumour (*n* = 8)	NED 0	5.9	Watchful waiting	6	–	5	–	1
	AWD 7		Surgery	1	–	1	–	–
	DFD 0		RT	1	–	1	–	–
	LTF 1		Surgery + adjuvant RT	–	–	–	–	–

NED, no evidence of disease; *AWD*, alive with disease; *DFD*, died from disease; *LTF*, lost to follow-up; *RT*, radiotherapy.

Nine of 11 patients with a solitary carotid body tumour showed no evidence of disease after surgery; the other two patients were lost to follow-up. Of five patients with a solitary jugular body tumour who underwent surgery, four received adjuvant radiotherapy although tumour-free margins were never achieved at resection. Only two of eight patients with a vagal body PGL received a form of treatment (1 radiotherapy and 1 surgery), and seven of these patients were alive with disease at the end of follow-up.

6.5 DISCUSSION

This study describes patients with HNPGL identified from a nationwide cohort of *SDHB* mutation carriers. The mean age at diagnosis of an HNPGL in this cohort (45·9 years) was higher than that reported previously, of between 30 and 37 years[8,10,18]. In the Netherlands, tumour screening in *SDHB*-linked families is advised from the age of 18 years onwards. A later start for tumour screening has been proposed based on statistical models of the age-dependent penetrance of *SDHB* mutations and, although the mean age in this cohort was relatively high, the youngest patient developed an HNPGL at age 11 years, and an 18-year-old patient had already developed PGL metastases. The optimal age to start screening for PGLs in *SDHB* mutation carriers thus remains a subject of debate[19-21].

The majority of patients in this cohort carried a Dutch *SDHB* founder mutation, either a deletion of exon 3 (18 of 54 patients) or the c.423+1G>A mutation (11 patients). Interestingly, the majority of patients with an *SDHB*-linked HNPGL reported a negative family history (70 per cent), probably reflecting the low penetrance of *SDHB*-linked PGL syndrome[22,23]. In addition, patients and their physicians may have been unaware that phaeochromocytomas and some non-paraganglionic tumours such as GISTs, pituitary tumours and renal clear cell carcinomas are part of the tumour spectrum caused by *SDHB* germline mutations[13].

Patients with *SDHB*-linked HNPGL had a low risk (8 of 54, 15 per cent) of developing multiple PGLs, in contrast to the risk for *SDHD* mutation carriers (60–79 per cent)[9,10,24]. Only a single patient in this *SDHB*-linked HNPGL cohort developed a phaeochromocytoma. Thirty-five years after an adrenalectomy for this tumour, this patient developed a vagal body tumour. No patient with an HNPGL developed extra-adrenal PGLs, even though these tumours are reported to be relatively prevalent in *SDHB* mutation carriers[12].

The risk of malignancy in this cohort was also lower than expected, with only three patients (6 per cent) developing metastases. All three presented with an

apparently benign solitary HNPGL (located in the tonsil, jugular body and carotid body). Metastatic disease developed during follow-up, at varying time intervals from initial HNPGL diagnosis (range 2·2–31·3 years). No clear clinical or genetic indicators of malignancy were identified.

Most patients with a carotid, jugular or tympanic body HNPGL had one or more complaints associated with the tumour (figure 6.1). Of the 13 patients (24 per cent) without symptoms, vagal body tumours dominated (over 50 per cent). The benefit of detecting asymptomatic, slow-growing benign PGLs through pre-symptomatic screening of *SDHB* mutation carriers is uncertain, as intervention by either surgery or radiotherapy may cause more morbidity than the tumour itself. Conversely, early diagnosis seems favourable in growing tumours, catecholamine-producing tumours and malignant tumours, allowing for timely therapeutic intervention. As the occurrence or type of symptoms does not reliably predict tumour growth, catecholamine excess or malignancy, adequate surveillance of *SDHB* germline mutation carriers is mandatory and should include screening for catecholamines or their metabolites, along with periodic radiological investigation of the abdomen, the pelvic region, thorax, and head and neck region. In patients with *SDHB*-linked HNPGLs, these regions should be evaluated not only for the occurrence of concurrent PGLs and phaeochromocytomas, but also for *SDHB*-associated non-paraganglionic tumours and PGL metastases.

The choice of an optimal treatment strategy for HNPGLs is complex and depends on diverse factors such as the causal gene mutation, patient characteristics (age, condition and preferences) and HNPGL characteristics (localization, size and growth rate, catecholamine excess and associated cranial nerve deficits). Opinions regarding adequate management of HNPGLs have changed over time and vary from centre to centre. Symptoms and risks conferred by the tumour should be weighed against the morbidity of the treatment. As the risk to *SDHB*-linked patients is not confined to one specific anatomical region or tumour type, these decisions are probably made most appropriately by a dedicated multidisciplinary team.

Genetic counselling and DNA testing is recommended for all patients with HNPGL, as different PGL-associated genes confer different clinical risks and may warrant different management strategies. If an *SDHB* germline mutation is identified in a patient with HNPGL, the clinician should be aware of the variable manifestations of the *SDHB*-linked tumour syndrome and, irrespective of the chosen management strategy, periodic surveillance should be performed including screening for catecholamine excess, concurrent PGL or phaeochromocytoma, metastatic PGL and *SDHB*-associated non-paraganglionic tumours.

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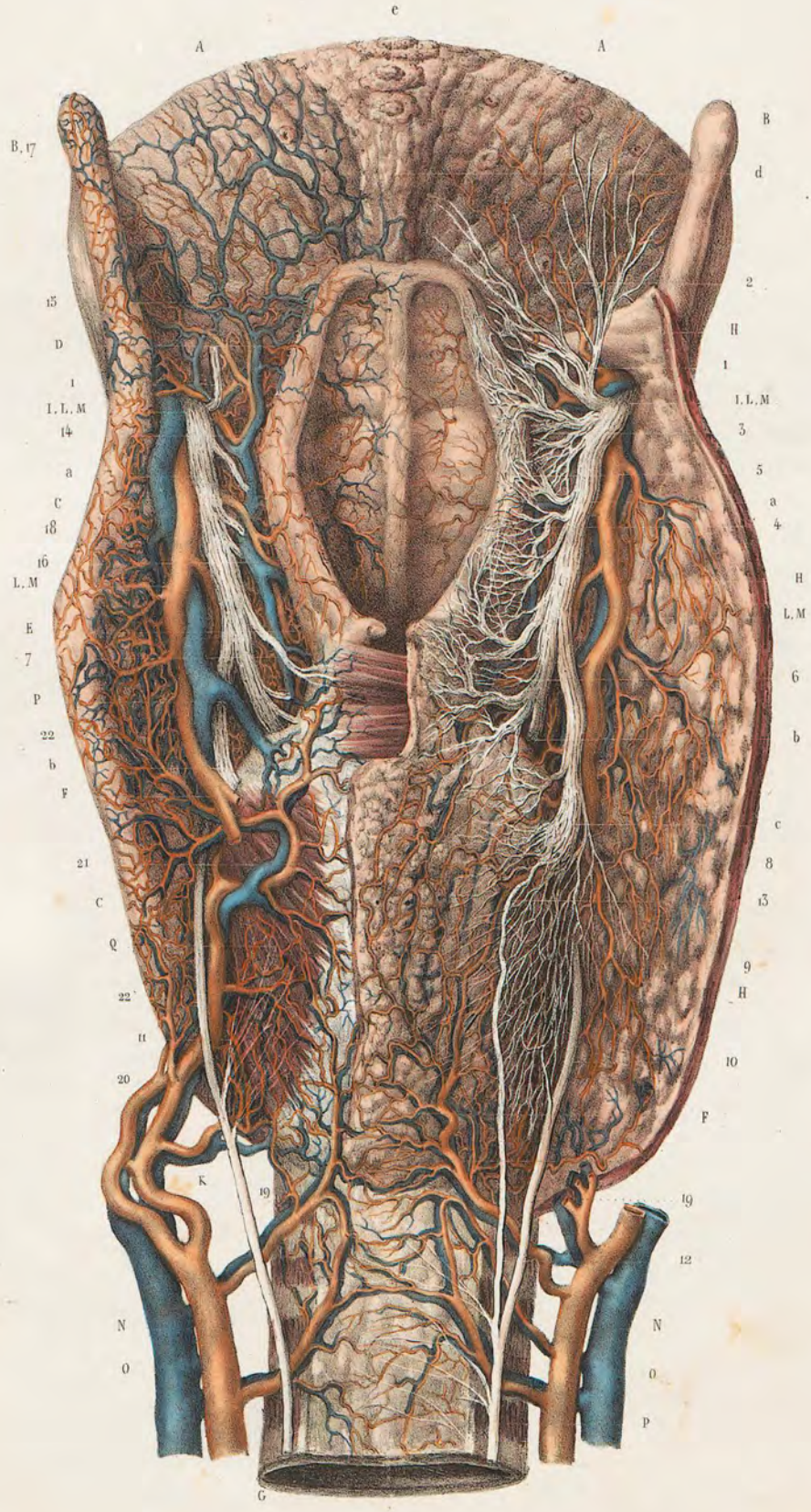
Fig. 1



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7 The penetrance of paraganglioma and pheochromocytoma in *SDHB* germline mutation carriers

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7.1 ABSTRACT

Germline mutations in succinate dehydrogenase B (*SDHB*) predispose to hereditary paraganglioma (PGL) syndrome type 4. The risk of developing PGL or pheochromocytoma (PHEO) in *SDHB* mutation carriers is subject of recent debate. In the present nationwide cohort study of *SDHB* mutation carriers identified by the clinical genetics centers of the Netherlands, we have calculated the penetrance of *SDHB* associated tumors using a novel maximum likelihood estimator. This estimator addresses ascertainment bias and missing data on pedigree size and structure. A total of 195 *SDHB* mutation carriers were included, carrying 27 different *SDHB* mutations. The 2 most prevalent *SDHB* mutations were Dutch founder mutations: a deletion in exon 3 (31% of mutation carriers) and the c.423+1G>A mutation (24% of mutation carriers). One hundred and twelve carriers (57%) displayed no physical, radiological or biochemical evidence of PGL or PHEO. Fifty-four patients had a head and neck PGL (28%), 4 patients had a PHEO (2%), 26 patients an extra-adrenal PGL (13%). The overall penetrance of *SDHB* mutations is estimated to be 21% at age 50 and 42% at age 70 when adequately corrected for ascertainment. These estimates are lower than previously reported penetrance estimates of *SDHB*-linked cohorts. Similar disease risks are found for different *SDHB* germline mutations as well as for male and female *SDHB* mutation carriers.

7.2 INTRODUCTION

Paragangliomas (PGLs) are rare, mostly benign neoplasms, which are embryologically derived from neural crest cells of the autonomic nervous system. PGL can be subdivided into head and neck paraganglioma (HNPGL), pheochromocytoma (PHEO) and thoracic and abdominal extra-adrenal PGL. PGLs can occur sporadically or as part of a hereditary syndrome. A rapidly expanding number of genes are associated with hereditary PGL/PHEO. Hereditary PGL syndrome is most frequently caused by genes encoding succinate dehydrogenase (*SDH*) subunits or cofactors, that is, *SDHA/B/C/D/AF2*. Other associated genes are *RET*, *NF1*, *VHL*, *HIF2A*, *FH*, *TMEM127* or *MAX*[1,2]. In the Netherlands, the majority of hereditary PGLs and a considerable number of seemingly sporadic tumors are caused by germline mutations in *SDHB* and *SDHD*. The mutation spectrum is dominated by a limited number of Dutch founder mutations, predominantly in *SDHD* but also in *SDHB* and *SDHAF2*[3]. In contrast to *SDHD* and *SDHAF2* mutation carriers, *SDHB* mutation carriers are reported to develop metastatic PGLs more frequently[4-9]. Germline mutations in *SDHB* are transmitted in an autosomal dominant way with incomplete penetrance. The reported penetrance of *SDHB* mutations varies widely (9%-75%) and is lower than the penetrance of (paternally inherited) *SDHD* or *SDHAF2* mutations (88%-100% and 87%-100%, respectively)[7,10-19]. The majority of earlier series on the penetrance of *SDHB* mutations are largely based on cohorts of symptomatic PGL patients and a limited number of asymptomatic family members. The resulting overrepresentation of affected individuals and the fact that close case relatives tend to share additional genetic and/or environmental risk factors may lead to an overestimation of disease risk (ascertainment bias). Recent single-family-based studies that involve a more comprehensive screening and surveillance of asymptomatic family members of index patients have shown lower penetrance estimates for *SDHB* mutations[18-20]. An important limitation of single-family studies is that the outcome is based on 1 specific *SDHB* mutation, and the results of penetrance calculations may not be representative for carriers of other *SDHB* mutations.

In addition to the limited inclusion of asymptomatic mutation carriers, the insights into adequate methodology for penetrance calculations have also progressed over time. In earlier penetrance studies, Kaplan-Meier estimators were used to describe the penetrance. In order to correct for the ascertainment bias, index patients were left out of the analysis. This method is prone to bias especially in low-penetrant disease, as it discards a relatively large amount of valuable information of affected mutation carriers. It furthermore does not actually correct for the way family members are ascertained, because the reason these family members

come under medical attention remains in the fact that they are part of a family with at least one affected family member.

Here, we present the age-related penetrance of *SDHB* mutations carriers identified in the clinical genetics centers in the Netherlands in a nationwide study. By the inclusion of *SDHB* mutation carriers using the registries of the clinical genetics centers, rather than through the tertiary referral centers that treat PGL patients, we were able to include carriers of germline mutations in *SDHB* in the Netherlands irrespective of their symptomatology. Moreover, we used a novel maximum likelihood estimator for the penetrance calculations, which allows for correction of the ascertainment bias and missing pedigree data using index patients, non-index patients and disease-free mutation carriers. In addition, we compare the penetrance estimates for different *SDHB* mutation subgroups and for male and female mutation carriers.

7.3 MATERIALS AND METHODS

SDHB germline mutation carriers were identified and included in this retrospective study by 7 of the 8 clinical genetics centers of 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). Maastricht University Medical Center was not able to participate for technical reasons. However, they only had identified 1 germline *SDHB* mutation carrier. The genotype and phenotype of this cohort, 61 *SDHB* mutation carriers from the University Medical Center Groningen and 47 *SDHB* mutation carriers from the Leiden University Medical Center have in part been described previously[9,21,22]. All included individuals participated in the study after written informed consent. In case of individuals under 18 years of age, written informed consent was obtained from their parents/guardians. 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.

Genetic counseling and DNA testing for mutations in the *SDHD*, *SDHB*, *SDHC* and *SDHAF2* gene are offered to patients with PHEO/extra-adrenal PGL and a positive family history for HNPGL or PHEO/extra-adrenal PGL, patients with an isolated PHEO/extra-adrenal PGL 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 was performed by direct sequencing using the Sanger method on an ABI 3777 Genetic Analyzer (Applied Biosystems, Carlsbad, California) and by multiplex ligation-dependent probe amplification (MLPA) using the P226 MLPA kit (MRC Holland, Amsterdam, the Netherlands). *SDHB* germline variants are classified as in the international guidelines by Plon *et al*.[23]. In this manuscript we report pathogenic or probably pathogenic variants, including missense mutations in highly conserved regions that are probably pathogenic, as germline mutations.

Mutation carriers were investigated for the occurrence of PGL and/or PHEO according to structured protocols used for standard care in the Netherlands for patients with a PGL or PHEO[24,25]. They were offered annual clinical surveillance for PGL/PHEO at the departments of otorhinolaryngology and endocrinology. For mutation carriers older than 18 years of age, surveillance consisted of magnetic resonance imaging (MRI) of the head and neck region once every 3 years, and MRI or computed tomography (CT) scans of thorax, abdomen and pelvis once every 2 years. Annual biochemical screening included the measurement of (nor) epinephrine, vanillylmandelic acid, dopamine, (nor)metanephrine and/or 3-methoxytyramine in 2 24-hour urinary samples (depending on the Academic Center which urinary measurement(s) were performed), and/or plasma free (nor) metanephrine. In case of excessive catecholamine secretion (ie, 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. In cases without available tumor histology, tumors were classified as paraganglionic based on their specific characteristics on CT and/or MRI. When in doubt, additional nuclear medicine imaging studies were performed in order to confirm the diagnosis. 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 HNPGL, PHEO or extra-adrenal PGL, intensified surveillance or treatment was offered. In general, for a PHEO or extra-adrenal PGL surgical resection was the preferred treatment option. In case of a HNPGL, the management strategy was guided by clinical symptoms, tumor characteristics such as localization, size and growth rate, and patient characteristics such as age, comorbidity and patient preferences. A wait and scan policy, radiotherapy or resection were possible treatment options.

Statistics

The cohort consisted of index patients (defined as PGL/PHEO patients with a negative family history) and their relatives (*SDHB* mutation carriers with a positive family history who were assumed to be identified via an index patient and not by the presence of a tumor indicative of PGL syndrome). All individuals in the data set carried a germline mutation in the *SDHB* gene. The maximum likelihood estimates were determined for the penetrance for all mutations together and for 3 genotypic subgroups separately (patients linked to a deletion exon 3, patients linked to the c.423+1G>A mutation and the remainder mutations in *SDHB*, respectively). In addition, maximum likelihood estimates were determined for males and females separately. The penetrance function was assumed to equal Weibull distributions with unknown shape and scale parameters. This novel method for the calculation of the penetrance function is described in more detail in the appendix. The likelihood ratio test was used to test for differences between the penetrance functions of males and females, and between the penetrance functions of the 3 genotypic subgroups. Based on the data of all *SDHB*-linked patients (index or non-index), the age-at-diagnosis distribution was estimated for all mutations together, for 3 genotypic subgroups separately, and for males and females separately, by their empirical distribution functions. The log rank test was used to test for differences between the age-at-diagnosis distributions of these subgroups.

IBM SPSS Statistics version 20.0 (SPSS Inc., Chicago, Illinois) and the statistical package R, version 3.0.1 were used for data analysis. Results were expressed as mean ± SD.

7.4 RESULTS

In all, 195 *SDHB* germline mutation carriers were identified and included in the study, 83 men (42.6%) and 112 women (57.4%), carrying 27 distinct *SDHB* mutations (table 7.1). The genotype and phenotype of this cohort have been described in detail elsewhere[21]. The most prevalent *SDHB* mutations were a deletion in exon 3 (61/195, 31.3% of mutation carriers) and the c.423+1G>A mutation (46/195, 23.6% of mutation carriers). The c.654G>A p.(Trp218*) mutation was present in 19 individuals (9.7%). Sixty-five carriers were index-patients, of whom 32 men (49.2%) and 33 women (50.8%). One hundred and thirty carriers (66.7%) had a positive family history for PGL. The mean age at presentation at the outpatient clinic was 44.6 ±16.3 years (range 2-76). In all, 83 of 195 (42.6%) *SDHB* germline mutation carriers were diagnosed with one or more PGL/PHEOs by CT and MRI (74/83; 89.2%), or by either CT or MRI and another (total body) imaging study (ie, ^{123}I MIBG-scans/^{18}F-FDG PET-scans/^{18}F-DOPA PET-scans) (9/83; 10.8%). At least 1 HNPGL was present in 54 of

Table 7.1 Germline mutations in *SDHB* in the Netherlands.

DNA mutation in *SDHB*	SDHB predicted protein change	Number of mutation carriers (n= 195)	Number of PGL/ PHEO patients (n=83)
exon 3 deletion	p.?	61	27
c.423+1G>A	p.?	46	16
c.654G>A	p.(Trp218*)	19	2
c.653G>C	p.(Trp218Ser)	11	4
c.574T>C	p.(Cys192Arg)	8	2
c.200+1G>A	p.?	6	4
c.137G>A	p.(Arg46Gln)	4	3
c.328A>C	p.(Thr110Pro)	4	2
c.418G>T	p.(Val140Phe)	4	1
c.725G>A	p.(Arg242His)	3	2
c.649C>T	p.(Arg217Cys)	3	1
c.590C>G	p.(Pro197Arg)	3	1
c.686_725del	p.(Glu229fs)	3	2
c.343C>T	p.(Arg115*)	3	3
c.292T>C	p.(Cys98Arg)	2	1
deletion promoter till exon 8	p.0	2	1
exon 2 deletion	p.?	2	1
exon 1 deletion	p.?	2	2
deletion promoter and exon 1	p.?	1	1
c.713delT	p.(Phe238fs)	1	0*
c.727T>A	p.(Cys243Ser)	1	1
c.761C>T	p.(Pro254Leu)	1	1
c.626C>T	p.(Pro209Leu)	1	1
c.380T>C	p.(Ile127Thr)	1	1
c.325A>C	p.(Asn109His)	1	1
c.1A>G	p.?	1	1
c.119A>C	p.(Lys40Thr)	1	1

Abbreviations: *PGL*, paraganglioma; *PHEO*, pheochromocytoma, *SDHB*, succinate dehydrogenase B.

* his individual underwent presymptomatic testing (PST) because of positive family history, however, the index patient of this individuals' family was not tested in the Netherlands.

195 patients (27.7%), in total 63 head and neck tumors were identified (mean age of diagnosis 45.9 ±14.1 years [range 11-77]). Four patients (2.1%) had a PHEO (mean age of diagnosis 36.2 ±16.3 years [range 19-56]), 26 patients (13.3%) had at least 1 extra-adrenal PGL (31 tumors in total) (mean age of diagnosis 34.4 ±12.7 years [range 19-56]) and 15 patients (7.7%) had a malignant PGL, defined as metastatic PGL in non-PGL tissue. The mean duration of follow up was 4.4 ± 2.6 years (range 0-36). One hundred and twelve carriers (57.4%) displayed no physical, radiological or biochemical evidence of PGL or PHEO. Complete radiological screening (CT/MRI

of the head and neck region and CT/MRI of the thorax/abdomen/pelvis) was available in 105 of 112 (93.8%) clinically unaffected mutation carriers. Two mutation carriers (2/112; 1.8%) underwent a CT and/or MRI of the head and neck region only, 5 of 112 (4.5%) underwent CT and/or MRI of the thorax/ abdomen/pelvis only.

Penetrance

The estimated penetrance of *SDHB* mutations for PGL and PHEO is shown in figure 7.1.

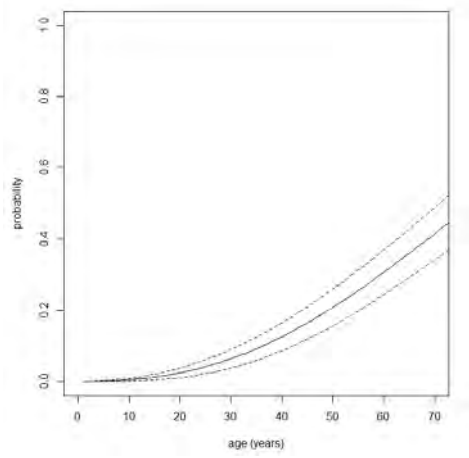

Figure 7.1 The maximum likelihood estimate of the age-related penetrance of *SDHB* mutations for paraganglioma and/or pheochromocytoma (continuous line) and 95% confidence interval (dashed line). The shape and scale parameters of the Weibull distribution are estimated as 2.50 and 89.1.

The overall penetrance is 0.064 at age 30 (confidence interval [CI] = 0.037-0.091), 0.126 at age 40 (CI = 0.087-0.166), 0.210 at age 50 (CI = 0.158-0.262), 0.311 at age 60 (CI = 0.248-0.374) and 0.421 at 70 years (CI = 0.348-0.495). We did not find a statistically significant difference in the penetrance between different *SDHB* mutations, that is, the Dutch *SDHB* exon 3 founder deletion, the Dutch *SDHB* founder mutation c.423 +1G>A, and the other mutations in *SDHB* grouped together (likelihood ratio test *P* = .740) (figure 7.2A). We also did not find a statistically significant difference in the *SDHB*-linked penetrance between male and female mutation carriers (likelihood ratio test *P* = .368) (figure 7.2B).

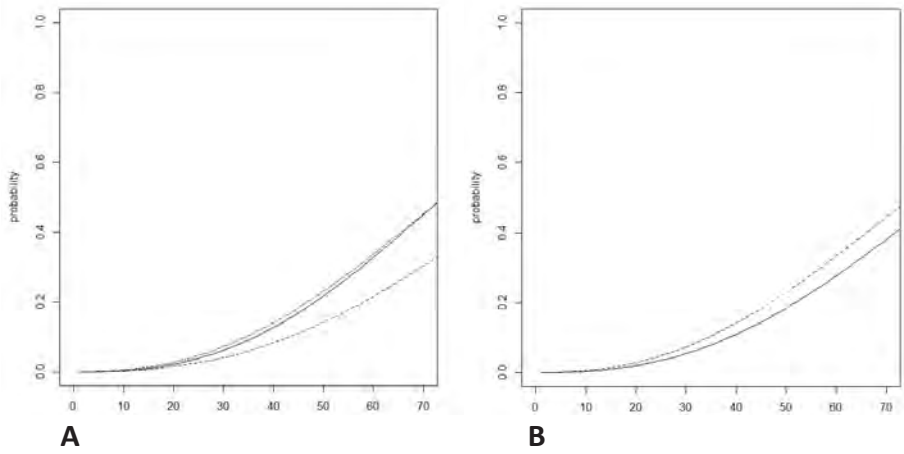

Figure 7.2 The maximum likelihood estimate of the penetrance for paraganglioma and/or pheochromocytoma. A, Age-related penetrance for different *SDHB* mutations. Continuous line: Dutch founder deletion in exon 3; dashed line: c.423+1G>A mutation; dash-dotted line: all other mutations in *SDHB* grouped together. Confidence intervals are left out for better visualization. B, Age-related penetrance for male and female *SDHB* mutation carriers. Continuous line: males; dashed line: females.

Age at diagnosis

The estimated age distribution for the diagnosis of the first index tumor of PGL/PHEO patients is shown in figure 7.3. Figure 7.4A shows the age at diagnosis for the 3 genotypic subgroups: PGL/PHEO patients linked to a deletion in exon 3, patients linked to the c.423 +1G>A mutation, and all remainder mutations in *SDHB*, respectively. We did not find a significant difference in the age at diagnosis between these genetic subgroups (log rank test *P* = .462). We also did not find a statistically significant difference in the age at diagnosis between male and female mutation carriers (log rank test P = .105) (figure 7.4B).

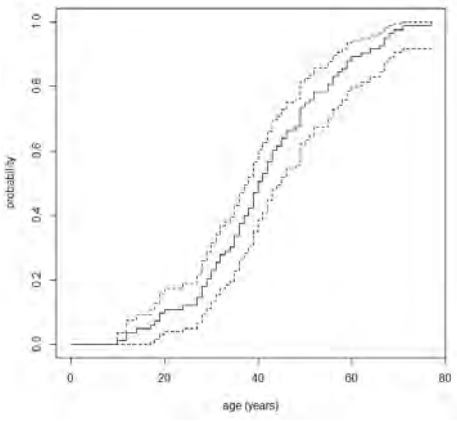

Figure 7.3 Estimated age at diagnosis of *SDHB*-linked paraganglioma syndrome (continuous line) and the confidence interval (dashed line).

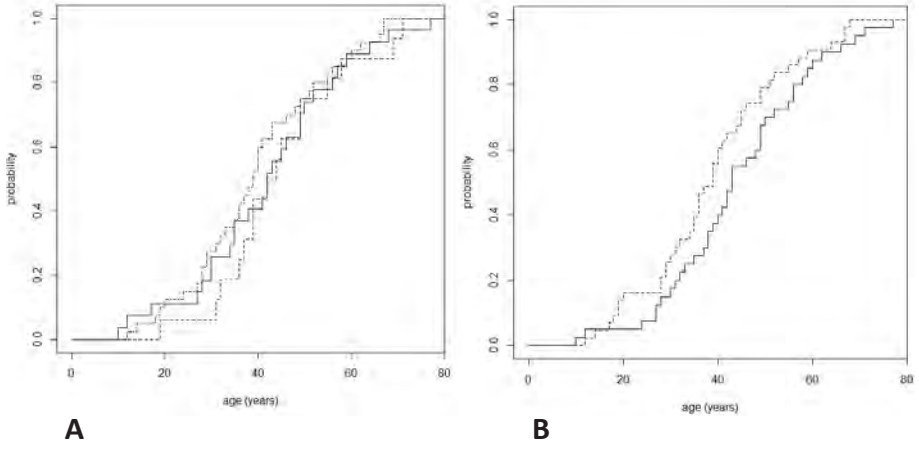

Figure 7.4 Estimated age at diagnosis of *SDHB*-linked paraganglioma syndrome for subgroups of mutation carriers. A, Estimated age at diagnosis for carriers of different *SDHB* mutations. Continuous line: Dutch founder deletion in exon 3; dashed line: c.423+1G>A mutation; dash-dotted line: all other mutations in *SDHB* grouped together. Confidence intervals are left out for better visualization. B, Estimated age at diagnosis for male and female paraganglioma/pheochromocytoma patients. Continuous line: males; dashed line: females (log rank test P = .105).

7.5 DISCUSSION

The risk of developing PGL/PHEO for *SDHB* germline mutation carriers has been the subject of recent debate, and varying penetrance estimates have been reported. Establishing the true penetrance of *SDHB* mutations is critical for proper counseling of *SDHB* germline mutation carriers. In this nationwide study, we found an estimated age-dependent *SDHB*-linked penetrance of 21% at 50 years.

In addition we have compared the penetrance of different *SDHB* mutation subgroups. We found no significant differences in the penetrance estimates of the genotypic subgroups, that is, the 2 most prevalent Dutch founder mutations (the c.423+1G>A splice site mutation and an exon 3 deletion) and all other *SDHB* mutations grouped together (Table 7.1). As both founder mutations represent different mutation types (a missense mutation and an exon deletion), these results therefore seem applicable to *SDHB* mutations in general (Figure 7.2A). We also did not find a statistically significant difference in penetrance for male and female *SDHB* mutation carriers, despite a reported higher incidence of PGL in women (Figure 7.2B)[1,26]. This reported higher incidence in women is therefore probably not a feature of *SDHB* mutations, but may be a feature of other PGL/PHEO susceptibility genes.

In earlier studies, an overall penetrance of *SDHB* mutations was estimated to be as high as 50%-75% at 50 years. These studies were largely based on affected, apparently non-familial individuals[4,7,13]. Family-based studies generally allow for more adequate identification of asymptomatic mutation carriers by means of family screening, and thus more accurate penetrance estimates for single mutations. Recent studies in *SDHB*-linked families report penetrance estimates of 9%-35% by the age of 50 years[18-20]. The disadvantage of studies of single families is the fact that those results are based on 1 specific *SDHB* mutation, and may not represent the penetrance of other *SDHB* mutations. In this study, comprising virtually all known *SDHB* mutation carriers in the Netherlands, carrying a total of 27 different *SDHB* mutations, a relatively large number of asymptomatic *SDHB* mutation carriers (112/195) could be included because of the acquisition through clinical genetics centers, rather than through medical centers. In this way, the ascertainment bias is decreased, resulting in a more accurate penetrance estimate for *SDHB* mutations.

In order to further reduce bias, we did not use a standard Kaplan-Meier estimator for the calculation of the penetrance function, a method used in most early reports on germline *SDHB* mutations[4,7,13]. These estimators are prone to overes-

timation because they do not adequately correct for the ascertainment bias[16]. The frequently used method for correcting this source of bias, that is, leaving the index patients out of the analysis, does not actually correct for the way the mutation carriers remaining in the analysis are ascertained. These mutation carriers are identified because they are family members of index patients, and thus are probably to share environmental and genetic risk factors. An additional disadvantage of leaving index patients out of the analysis, especially in rare and low-penetrant hereditary disease such as *SDHB*-linked PGL syndrome, is that a relatively large amount of valuable data of affected mutation carriers is discarded.

More recent studies have used a maximum likelihood methodology to correct for the ascertainment bias, reporting lower *SDHB*-linked penetrance estimates of 9%-13% at 50 years[16,20]. These studies were family-based, and thus were able to use information of the family pedigrees. In the current cohort-based study, the information on pedigree size and structure is largely missing. Only individuals that underwent genetic testing are included in the analysis, untested family members at risk or obligate mutation carriers could not be identified, a common disadvantage of studies that are not family-based. This may lead to overestimation of disease risk because it is probably that healthy individuals without complaints are less inclined to consent to genetic testing than individuals with signs or symptoms indicative of PGL syndrome. Individuals that did undergo genetic testing are probably to be predominantly members of the nuclear family of PGL patients, and case relatives tend to share possible additional genetic and/or environmental risk factors. Unfortunately, it is not possible to reliably assess the number of individuals at risk who did not undergo genetic analysis.

We designed a maximum likelihood estimator to quantify the effect of the missing data on the pedigree size and structure on penetrance estimates (see appendix). This statistical method is not a substitute for thorough family screening and pedigree analysis (ideally a prospective analysis of large multigenerational *SDHB*-linked kindreds with detailed pedigree information, complete genetic screening and clinical surveillance, and complete follow up), in fact it uses what information there is and yields more accurate results with smaller confidence intervals when more pedigree information is available. It allows for the quantification of the effect of the missing data on the penetrance estimations in situations where pedigree data are (partly) missing, using the data of all affected and unaffected *SDHB* germline mutation carriers, including index patients. Using this maximum likelihood estimator on our nationwide cohort of *SDHB* mutation carriers (including a relatively large number of disease-free mutation carriers), our penetrance estimates are lower than previously reported in cohort-based studies.

In conclusion, the current best estimate of the penetrance of *SDHB*-linked PGL/PHEO syndrome is 21% at 50 and 42% at 70 years of age. We find no difference in the penetrance of PGL and PHEO between different *SDHB* germline mutations, nor between male and female *SDHB* mutation carriers.

7.6 REFERENCES

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7.7 APPENDIX

1 Estimation of the penetrance

We estimate the penetrance by the maximizing likelihood estimator; the function that maximizes the likelihood. In this appendix, we describe the likelihood, the assumptions we make, as well as how to test for differences in penetrance for males and females and for mutational subgroups. We start with introducing some notation.

We denote the age at onset of the disease by T, and the age at the end of the study or the age at death not due to the disease, which one comes first, by C. Furthermore, we assume that T and C are stochastically independent. For every individual we observe whether he/she is an index patient or a relative of an index patient, but information on the pedigree structure is not observed. For individuals with the disease we always observe T and C (unless the individual died due to the disease during the study). For the individuals without the disease, we observe C, but not T. The function Δ equals 1 if the individual has the disease and 0 otherwise. In order to distinguish between individuals we use an underscore: T_i, C_i and Δ_i for individual i.

We assume that the distribution for T, the penetrance of interest, denoted as F_θ with density f_θ, is the Weibull distribution with unknown (two-dimensional) parameter θ. The distribution for C is also unknown and we denote it as G.

We assume that the phenotypic data of individuals (mutation-carriers) are independent (so conditional they carry the mutation, their phenotypes are independent). Under this assumption, the conditional likelihood (conditional the ascertainment event that at least one of the family members has the disease) is proportional to

$$L \propto \frac{\prod_{i=1}^{n} f_\theta(T_i)^{\Delta_i}(1-F_\theta(C_i))^{1-\Delta_i}}{\prod_{j=1}^{r} 1-\left(\int 1-F_\theta(s)dG(s)\right)^{n_j}},$$

where n equals the number of mutation-carriers and r the number of pedigrees in the data-set (i.e. the number of index patients), with $n > r$, and n_j is the number of individuals in pedigree j. Since the pedigree sizes, n_j, are unknown and it is not possible to estimate them from the data, we replace them by the average pedigree size in the data-set, so by $\sum_{j=1}^{r} n_j/r = n/r$.

The distribution G is unknown. We estimate this distribution by the empirical distribution function of the censoring times (C_i) of the relatives only. Next we insert this estimated function into the likelihood and maximize it with respect to the parameter θ by a grid-search, to obtain the maximum likelihood estimate for F_θ. Confidence intervals for θ and F_θ are constructed with help of the asymptotic normality of maximum likelihood estimators, the Delta-method and the main theorem proofed in Jonker et al (2014). More details of this, the construction of the likelihood and the estimation of θ can be found in Jonker et al (2016).

The penetrance is also estimated for three genetic subgroups and for males and females separately. To test whether the penetrances differ for the genetic subgroups or for males and females, a likelihood ratio test is performed. The p-values are computed under the assumption that the likelihood ratio statistic is asymptotically chi-squared distributed. This is true if the distribution G is known. However, when constructing the confidence interval for θ we have seen that not knowing G hardly affects the accuracy. The computed p-values are probably only slightly too low, since the variability of the estimate for G is not taken into account.

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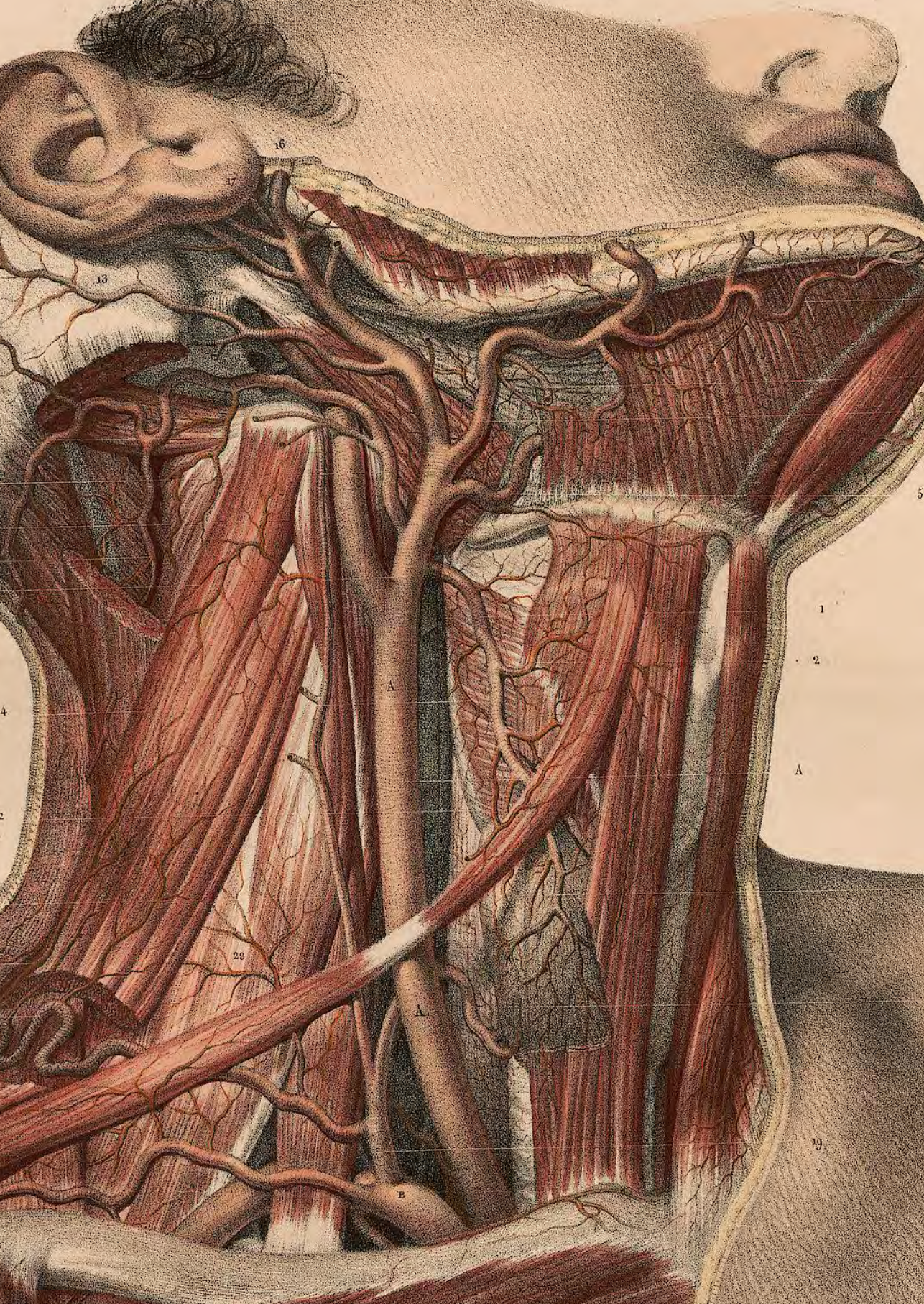
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8 Increased mortality in *SDHB* but not in *SDHD* pathogenic variant carriers

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Cancers (Basel). 2019 Jan 17;11.

8.1 ABSTRACT

Germline mutations in succinate dehydrogenase subunit B and D (*SDHB* and *SDHD*) are predisposed to hereditary paraganglioma (PGL) and pheochromocytoma (PHEO). The phenotype of pathogenic variants varies according to the causative gene. In this retrospective study, we estimate the mortality of a nationwide cohort of *SDHB* variant carriers and that of a large cohort of *SDHD* variant carriers and compare it to the mortality of a matched cohort of the general Dutch population. A total of 192 *SDHB* variant carriers and 232 *SDHD* variant carriers were included in this study. The Standard Mortality Ratio (SMR) for *SDHB* mutation carriers was 1.89, increasing to 2.88 in carriers affected by PGL. For *SDHD* variant carriers the SMR was 0.93 and 1.06 in affected carriers. Compared to the general population, mortality seems to be increased in *SDHB* variant carriers, especially in those affected by PGL. In *SDHD* variant carriers, the mortality is comparable to that of the general Dutch population, even if they are affected by PGL. This insight emphasizes the significance of DNA-testing in all PGL and PHEO patients, since different clinical risks may warrant gene-specific management strategies.

8.2 INTRODUCTION

Paragangliomas (PGL) are rare tumors that originate from cells of neural crest origin in the paraganglia associated with the autonomic nervous system. PGL can be subdivided into head and neck paragangliomas (HNPGL), pheochromocytomas (PHEO), and thoracic and abdominal extra-adrenal PGL (sympathetic PGL; sPGL). An increasing number of genes are associated with hereditary PGL/PHEO. Most frequently, hereditary PGL syndrome is caused by genes encoding subunits or cofactors of succinate dehydrogenase (*SDH*), such as *SDHA*/*B*/*C*/*D*/*AF2*. Other associated genes are *RET*, *NF1*, *VHL*, *HIF2A*, *FH*, *TMEM127*, and *MAX*[1,2]. In the Netherlands, pathogenic variants in *SDHD* are the most prevalent cause of PGL syndrome, followed by variants in *SDHB* and *SDHA*[3,4]. Although all *SDHx* genes encode subunits of the same *SDH* complex and pathogenic variants all disrupt its enzymatic function, different genes are associated with different phenotypes. The reported lifelong penetrance of pathogenic *SDHB* variants (22–42%)[5,6] is considerably lower than the penetrance of paternally inherited *SDHD* mutations (88–100%)[7–10].

When pathogenic *SDHB* variants cause disease, the clinical outcome is reported to be less favorable than that in *SDHD*-linked disease. *SDHB* mutation carriers are reported to develop metastatic PGL more frequently and patients with metastatic disease associated with *SDHB* variants are reported to have a poor 5-year survival rate compared to patients with metastatic disease associated with other causative genes[11]. The mortality of *SDHB* variant carriers is currently unknown[12]. In this study we estimate the mortality for a nationwide cohort of *SDHB* variant carriers and compare this risk with the mortality of *SDHD* variant carriers and that of the general Dutch population.

8.3 SUBJECTS AND METHODS

Eligibility Criteria

The cohort of pathogenic germline variant carriers (hereafter variants) in *SDHB* included in this study has been described in detail previously[6,13]. The mortality of this nationwide *SDHB*-linked cohort was compared with the mortality of the general Dutch population and with the mortality of an updated cohort of *SDHD* variant carriers, which has been described previously[12]. Only *SDHD* variant carriers with paternal inheritance were included. Carriers of *SDHD* variants were identified using the database of the Laboratory for Diagnostic Genome Analysis (LDGA) at the Leiden University Medical Center (LUMC), a tertiary referral center

for patients with PGL. Screening for *SDH* variants was performed in all persons diagnosed with PGL who agreed to genetic testing.

Screening for *SDHB* and *SDHD* variants was performed by direct sequencing of peripheral blood leucocytes using the Sanger method on an ABI 377 Genetic Analyzer (Applied Biosystems, Carlsbad, California) and by multiplex ligation-dependent probe amplification (MLPA) using the P226 MLPA kit (MRC Holland, Amsterdam, the Netherlands). Family members of index patients were tested for the family-specific variant. All variants described in this study were submitted to the Leiden Open (source) Variation Database LOVD database (http://chromium.liacs.nl/lovd_sdh). *SDHB* and *SDHD* germline variants were classified according to the international guidelines put forth by Plon *et al.*[14]. *SDHD* variants were described using the reference sequence NG_012340.1 covering SDHB transcript NM_003000.2, and NG_012337.1 covering SDHD transcript NM_003002.2, available from the TCA Cycle Gene Variant Database LOVD database. In this manuscript we report pathogenic or likely pathogenic variants, including missense mutations in highly conserved regions that are determined to be likely pathogenic as germline mutations based partly on mutation prediction analyses. Information on amino acid conservation can be found in the LOVD database (http://chromium.liacs.nl/lovd_sdh). Further information including mutation prediction analyses can be obtained on request.

The study was approved by the Medical Ethics Committee of the Leiden University Medical Center; participating centers complied with their local Medical Ethics Committee requirements. Written informed consent was obtained from the parents/guardians of individuals under 18 years of age.

Clinical Characteristics

Clinical data were retrieved from medical records. Pathogenic variant carriers were investigated for occurrences of PGL and/or PHEO according to the structured protocols used for standard care in the Netherlands for PGL or PHEO patients[15,16]. Patients were offered clinical surveillance for PGL/PHEO at the departments of otorhinolaryngology and endocrinology. For asymptomatic *SDHB* and *SDHD* variant carriers older than 18 years of age, surveillance consisted of magnetic resonance imaging (MRI) of the head and neck region once every 2–3 years, and MRI or computed tomography (CT) scans of the thorax, abdomen, and pelvis once every 1–2 years in *SDHB* variant carriers. Biochemical screening was performed annually on *SDHB* variant carriers, and every 1–2 years on *SDHD* variant carriers. This screening measured levels of (nor)epinephrine, vanillylmandelic acid, dopamine, (nor)metanephrine, and/or 3-methoxytyramine in two 24-hour urinary samples

(depending on the Academic Center in which urinary measurement(s) were performed), and/or plasma free (nor)metanephrine and 3-methoxytyramine. In cases of excessive catecholamine secretion (i.e., any value above the upper reference limit), radiological assessment by MRI or CT scans of the 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, 18F-L-dihydroxyphenylalanine (^{18}F-DOPA) PET-scans, or positron emission tomography with 1,4,7,10-tetraazacyclododecane-NI, NII, NIII, NIIII-tetraacetic acid (D)-Phe1-thy3-octreotide (^{68}Ga-DOTATOC PET) scans were performed to identify potential sources of excessive catecholamine production. In cases without available tumor histology, tumors were classified as paraganglionic based on their specific characteristics in CT and/or MRI scans. When in doubt, additional nuclear medicine imaging studies were performed in order to confirm the diagnosis. 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 HNPGL, PHEO or sPGL, intensified surveillance or treatment was offered. Surgical resection was generally the preferred treatment option for PHEO or sPGL. In cases of HNPGL, the management strategy was guided by clinical symptoms, tumor characteristics such as localization, size, and growth rate, and patient characteristics such as age, comorbidity, and patient preferences. A wait and scan policy, radiotherapy, or surgical resection were possible treatment options.

Mortality and Survival

For this study, follow-up data from *SDHB* and *SDHD* variant carriers were included from the date of the DNA test. In cases where clinical follow-up was available for the period before the DNA test, this period was not considered in the mortality analysis because it would have introduced immortal time bias[17]. Follow-up was defined as the time between the DNA test and the last clinical follow-up date before the end of the study period. Patients who were alive at the last clinical follow-up were classified as alive. Follow-up ended at the end of the study period, at the date of death or, in case of emigration, at the date of emigration[13]. To compare mortality between *SDHB* and *SDHD* variant carriers and the general population, the standardized mortality ratio (SMR) was estimated. Mortality rates for the Dutch population were obtained from Statistics Netherlands (CBS, The Netherlands)[18], using rates stratified by sex, age (per 1 year) and date (1-year periods). The SMR was calculated by dividing the observed number of deaths in the *SDHB* and *SDHD* cohorts.

The expected number of deaths was calculated as the sum of the stratified number of expected deaths (stratum-specific mortality rates from the general population times follow-up time at risk).

Survival was graphically displayed for *SDHB* and *SDHD* variant carriers by plotting survival in the carriers against the expected survival based on matched data from the general population. STATA 14.0 (Stata Corp, Texas, USA) was used for statistical analysis.

8.4 RESULTS

In total, 192 *SDHB* variant carriers and 232 *SDHD* variant carriers were included in this study. The clinical characteristics are depicted in table 8.1.

Table 8.1 Clinical characteristics of carriers of pathogenic variants in succinate dehydrogenase subunits B and D (*SDHB* and *SDHD*).

Clinical characteristics	*SDHB* *n* = 192	*SDHD* *n* = 232
Male (%)/female (%)	81 (42.2)/111 (57.8)	123 (53.0)/109 (47.0)
Mean age at genetic testing	46 years (range 9–77)	44 years (range 16–73)
HNPGL (%)	53 (27.6)	198 (85.3)
sPGL (%)	26 (13.5)	18 (7.8)
Pheochromocytoma (%)	4 (2.1)	16 (6.9)
Malignant PGL (%)	14 (7.3)	4 (1.7)
Unaffected (%)	110 (57.3)	30 (12.9)

HNPGL = head and neck paraganglioma, *sPGL* = sympathetic paraganglioma, *PGL* = paraganglioma.

The mean age at identification of the pathogenic gene variant was 46 years (range 9–77) in *SDHB* variant carriers and 44 years (range 16–73) in *SDHD* variant carriers. In total, 53 *SDHB* variant carriers (27.6%) and 198 *SDHD* variant carriers (85.3%) were diagnosed with HNPGL, either at time of presentation or during follow-up. Four *SDHB* patients (2.1%) and 16 *SDHD* patients (6.9%) developed PHEO and 26 *SDHB* patients (13.5%) and 18 (7.8%) *SDHD* patients developed sPGL. Malignant PGL, defined as metastatic PGL in non-paraganglionic tissue, were diagnosed in 14 *SDHB* (7.3%) and four *SDHD* patients (1.7%). Most *SDHB* variant carriers (110/193; 57.3%) were not affected at the time of DNA testing or during follow-up. In contrast, the majority of *SDHD* variant carriers was diagnosed with *SDHD*-associated disease (203/232; 87.5%). Details of the specific *SDHB* and *SDHD* variants are included in the appendix.

Mortality and SMR

Mortality data were available for all *SDHB* and *SDHD* variant carriers. The mean follow-up period was 3.0 (range 0–14.5) and 5.1 (range 0–12.5) years, respectively, for *SDHB* and *SDHD* variant carriers. In total, 6/192 (3.1%) *SDHB* variant carriers died at age 32, 37, 49, 52, 62, and 63. In three patients the cause of death was directly related to progressive PGL disease. In contrast, 5/232 (2.2%) *SDHD* variant carriers died at age 41, 43, 71, 71, and 74. In two cases the cause of death was most likely associated with PGL disease. Clinical characteristics of the variant carriers who died during the study period are listed in table 8.2.

A direct comparison between *SDHB* and *SDHD* variant carriers is hampered by the limited number of carriers and the heterogeneity between both groups. We performed an adjusted Poisson regression, adjusting for age, sex, and calendar time. The rate ratio comparing *SDHB* to *SDHD* variant carriers was 0.48 (95% confidence interval (CI) 0.15–1.62). However, the power for this analysis is low. As both groups have few events, we cannot draw conclusions from the non-significant þ-value.

For the comparison of both the *SDHB*- and *SDHD*-linked cohorts with normative data of the Dutch population, a total of 1781 person-years were available (*SDHB* 590 and *SDHD* 1191 years, respectively). The SMR for *SDHB* mutation carriers was 1.89 (95% confidence interval (CI) 0.85–4.21) (figure 8.1). A separate analysis including only symptomatic *SDHB* variant carriers- i.e., those with manifest disease - showed a higher SMR at 2.88 (95% CI 1.08–7.68). These results suggest an increased mortality risk for *SDHB* variant carriers compared to the general Dutch population, especially for carriers affected by *SDHB*-associated disease. For *SDHD* variant carriers, the SMR was 0.93 (95% CI 0.39–2.23), increasing only slightly to 1.06 (95% CI 0.44–2.54) in affected carriers, suggesting that mortality is not increased in *SDHD* variant carriers.

Table 8.2 Details of six *SDHB* and five *SDHD* variant carriers who died during follow-up.

Sex	Mutation	Predicted Protein Change	Location of PGL	Age at PGL Diagnosis (years)	Age at Diagnosis of Malignant Disease (years)	Age at Death (years)	Location of Metastases	Cause of Death
M	*SDHB* exon 3 deletion	p.?	Presacral	28	28	32	Bone	Progressive malignant PGL
F	*SDHB* c.654G > A	p.(Trp218*)	Bladder	19	58	62	Lymph nodes, bone	Progressive malignant PGL
F	*SDHB* exon 3 deletion	p.?	Para-vertebral abdominal	33	33	37	Lymph nodes, bone	Progressive malignant PGL
F	*SDHB* c.727T > A	p.(Cys243Ser)	Retroperitoneal (para-aortic)	52	55	63	Bone	Myocardial infarction, heart failure and acute respiratory distress syndrome
F	*SDHB* c.423 + 1G > A	p.?	n.a.	49	n.a.	52	n.a.	Respiratory insufficiency due to lung bleeding after chemoradiotherapy for lung cancer
F	*SDHB* c.423 + 1G > A	p.?	n.a.	42	n.a.	49	n.a.	Metastatic breast cancer
F	*SDHD* c.274G > T	p.(Asp92Tyr)	Bladder	42	42	43	Lymph nodes, bone marrow	Progressive malignant PGL
F	*SDHD* c.274G > T	p.(Asp92Tyr)	Mediastinal	67	67	74	Lymph nodes, bone	Unknown, however the patient was known to have progressive malignant PGL
F	*SDHD* c.274G > T	p.(Asp92Tyr)	Bilateral CBT, VBT	55	n.a.	71	n.a.	Cardiac arrest
F	*SDHD* c.242C > T	p.(Pro81Leu)	CBT	38	n.a.	41	n.a.	Breast cancer
M	*SDHD* c.274G > T	p.(Asp92Tyr)	CBT, jugular PGL, retroperitoneal	52	n.a.	71	n.a.	Prostate cancer

PGL = paraganglioma, *CBT* = carotid body tumor, *VBT* = vagal body tumor, *n.a.* = not applicable.

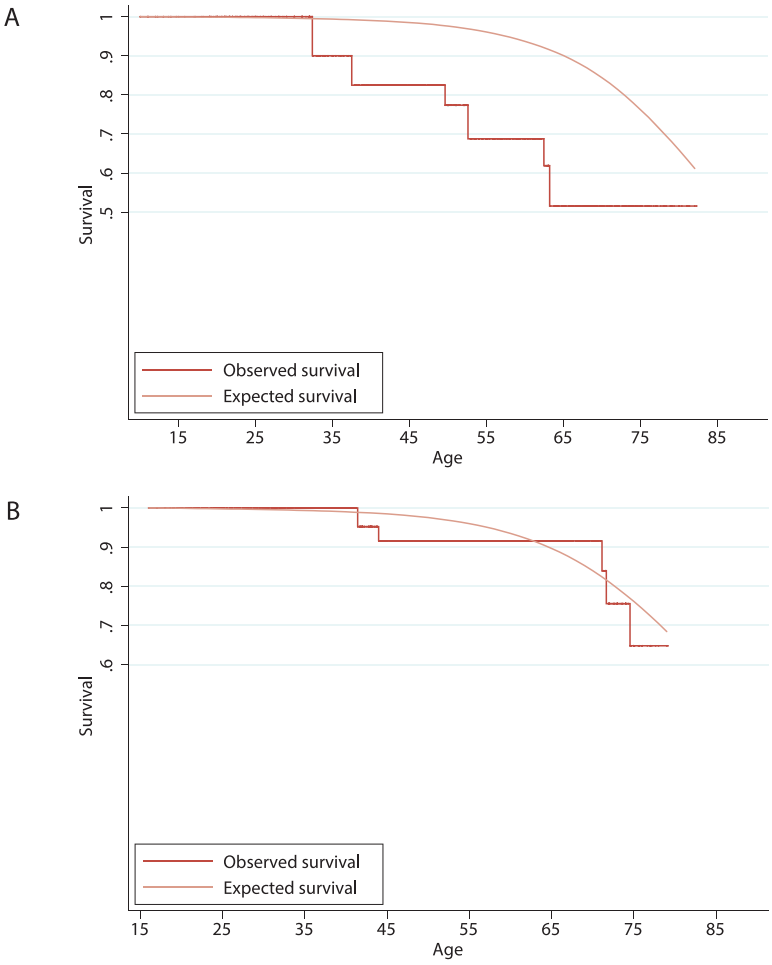

Figure 8.1 The Kaplan–Meier survival curve for *SDHB* variant carriers (A) and *SDHD* variant carriers (B) compared with the expected survival based on the general Dutch population.

8.5 DISCUSSION

In this study we estimated the mortality for *SDHB* and *SDHD* pathogenic variant carriers. Whereas the mortality for *SDHD* variant carriers is comparable with a matched cohort of the general Dutch population (SMR = 0.93), *SDHB* variant carriers show a higher mortality (SMR = 1.89, meaning a 1.89 times higher risk of death than the matched cohort of the general Dutch population).

These mortality ratios should be interpreted with some caution. First, not all deaths in our cohort are directly attributable to PGL-linked disease. However, a comparison is made with the mortality of the general Dutch population. Therefore, eliminating other causes of death would be inappropriate.

Second, even though the *SDHB* variant carriers represent a nationwide cohort, PGL is a rare disease and patient numbers are inevitably limited. As a result, the study estimates have broad confidence intervals. In addition, the follow-up of the start of this study is defined as the time of DNA testing and not PGL/PHEO diagnosis. As the genetic causes of hereditary PGL syndromes have been determined only recently, follow-up is relatively limited. However, the differences between *SDHB* and *SDHD* variant carriers are remarkable, all the more so when considering that *SDHD* variants are characterized by a high penetrance of PGL (88–100%), and *SDHB* variants by a much lower lifelong PGL risk (22–42%)[5–10]. In *SDHD* variant carriers, the occurrence of often multiple associated (HN)PGL seems to have no clear impact on survival[12]. In contrast, *SDHB* variant carriers seem to face increased mortality even though they are under more intensive surveillance and, in our study, have a shorter follow-up. This decreased survival of *SDHB* variant carriers is attributable to the higher mortality of affected *SDHB* patients (SMR = 2.88). Moreover, the majority of deceased *SDHB*-linked patients suffered from progressive malignant PGL (Table 8.2). Unaffected *SDHB* variant carriers have a mortality ratio that is more in line with the general Dutch population (SMR = 1.12).

It is intriguing that the causative gene seems to determine variation in the prognoses for PGL/PHEO patients, even though pathogenic variants in *SDHB* and *SDHD* cause PGL/PHEO syndrome through defects in the same protein complex (succinate dehydrogenase, *SDH*). We speculate that this could be the result of intrinsic properties of the *SDHB*-associated PGL/PHEO syndrome, a deleterious effect of *SDHB* variants on other factors that influence survival, or differences between *SDHB* and *SDHD* variants in the potential to induce other types of malignancy. Interestingly, other types of malignancies (i.e. prostate cancer, lung cancer, breast cancer) are listed as causes of death both in the *SDHB*- and *SDHD*-linked cohorts (see table 8.2). Although the *SDHx*-associated tumor spectrum is expanding, none of these malignancies have been directly linked to *SDHB* or *SDHD* variants. Even so, *SDHD* and/or *SDHB* variants could alter the susceptibility to certain types of malignancy other than PGL/PHEO. Indeed, 0.25% and 0.05% of breast cancer exomes carry somatic *SDHB* and *SDHD* variants, respectively[19,20].

The finding that all deceased *SDHB*-related PGL patients had metastatic PGL suggests that the occurrence of metastatic disease in *SDHB*-linked PGL syndrome

particularly impacts survival, and that metastases may be either more prevalent in *SDHB*-linked cases, as suggested before[7,10,21–24], or more aggressive than metastatic diseases associated with other *SDHx* genes, a finding that is in line with the very poor 5-year survival rate of *SDHB*-linked metastatic disease reported by Amar *et al.*[11]. Another explanation might be that metastases from sPGL behave more aggressively than those of parasympathetic HNPGL, and that these sPGL are more prevalent in *SDHB*-linked disease[13,25]. Indeed, the PGL patients that died of progressive PGL disease both in the *SDHB*- and *SDHD*-linked cohorts all suffered from primary sPGL tumors.

The difference in the mortality between *SDHB* and *SDHD* variant carriers is another clear indication that causative genetic alteration is of critical importance to the outcome and risks of an individual PGL patient. This is important in counseling PGL/PHEO patients, but may also warrant gene-specific management strategies for PGL patients. In the present study, however, we did not evaluate the effect of PGL follow-up protocols or treatment on survival. From the patients that died of *SDHB*-related disease (n = 3), two already had proven metastatic disease at the time of diagnosis. Surgical resection with tumor-free margins seems to be a logical treatment strategy when trying to avoid progression of the disease, but there may be undetected metastases already present at the time of surgery[26,27]. The observation that the higher mortality associated with *SDHB* variant carriers seems to be attributable to patients that are affected by metastatic sPGL may warrant a more aggressive surgical strategy towards sPGL tumors in *SDHB*-linked patients. The risk of the malignant transformation of an sPGL tumor left untreated is, however, unknown. This unknown risk of disease progression must be weighed against the risk of surgical morbidity[28].

8.6 CONCLUSION

In conclusion, compared to a matched cohort of the general population, mortality is increased in *SDHB* variant carriers but not in *SDHD* variant carriers. This insight emphasizes the significance of DNA-testing; gene-specific clinical risks may warrant tailored management strategies. Further research is necessary to demonstrate the effect of (early) intervention of PGL/PHEO on mortality rates, especially in *SDHB* variant carriers.

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8.8 APPENDIX

DNA Mutation	Predicted Protein Change	Number of Subjects (%)
Exon 3 deletion	p.?	59 (30.7)
c.423 + 1G > A	p.?	45 (23.4)
c.654G > A	p.(Trp218*)	19 (9.9)
c.653G > C	p.(Trp218Ser)	11 (5.7)
c.574T > C	p.(Cys192Arg)	8 (4.2)
c.200 + 1G > A	p.?	6 (3.1)
c.137G > A	p.(Arg46Gln)	4 (2.1)
c.328A > C	p.(Thr110Pro)	4 (2.1)
c.418G > T	p.(Val140Phe)	4 (2.1)
c.725G > A	p.(Arg242His)	3 (1.6)
c.649C > T	p.(Arg217Cys)	3 (1.6)
c.590C > G	p.(Pro197Arg)	3 (1.6)
c.686_725del	p.(Glu229fs)	3 (1.6)
c.343C > T	p.(Arg115*)	3 (1.6)
c.292T > C	p.(Cys98Arg)	2 (1.0)
Deletion promoter and exon 1	p.?	1 (0.5)
Deletion promoter till exon 8	p.0	2 (1.0)
Exon 2 deletion	p.?	2 (1.0)
Exon 1 deletion	p.?	2 (1.0)
c.713delT	p.(Phe238fs)	1 (0.5)
c.727T > A	p.(Cys243Ser)	1 (0.5)
c.761C > T	p.(Pro254Leu)	1 (0.5)
c.626C > T	p.(Pro209Leu)	1 (0.5)
c.380T > C	p.(Ile127Thr)	1 (0.5)
c.325A > C	p.(Asn109His)	1 (0.5)
c.1A > G	p.?	1 (0.5)
c.119A > C	p.(Lys40Thr)	1 (0.5)
c.274G > T	p.(Asp92Tyr)	175 (74.7)
c.416T > C	p.(Leu139Pro)	34 (14.6)
c.284T > C	p.(Leu95Pro)	6 (2.6)
Deletion promoter, exon 1 and 2	p.?	4 (1.7)
c.242C > T	p.(Pro81Leu)	3 (1.3)
c.337_340delGACT	p.(Asp113fs)	2 (0.9)
c.122dupC	p.(Glu42fs)	2 (0.9)
Exon 1. c.3G > C	p.(Met1Ile)	1 (0.4)
Exon 2: c.169_169 + 9del10, splice donor mutation	p.?	1 (0.4)
Intron 2 c.169_169 + 9del	p.?	1 (0.4)
Specific *SDHD* variant unknown (tested elsewhere)	unknown	3 (1.3)

SDHB variants and *SDHD* variants.

Fig. 1.

Fig. 4.

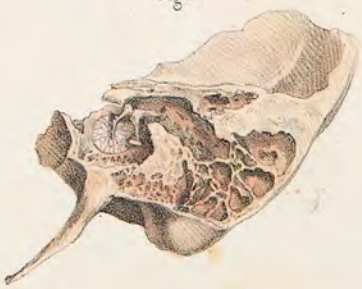
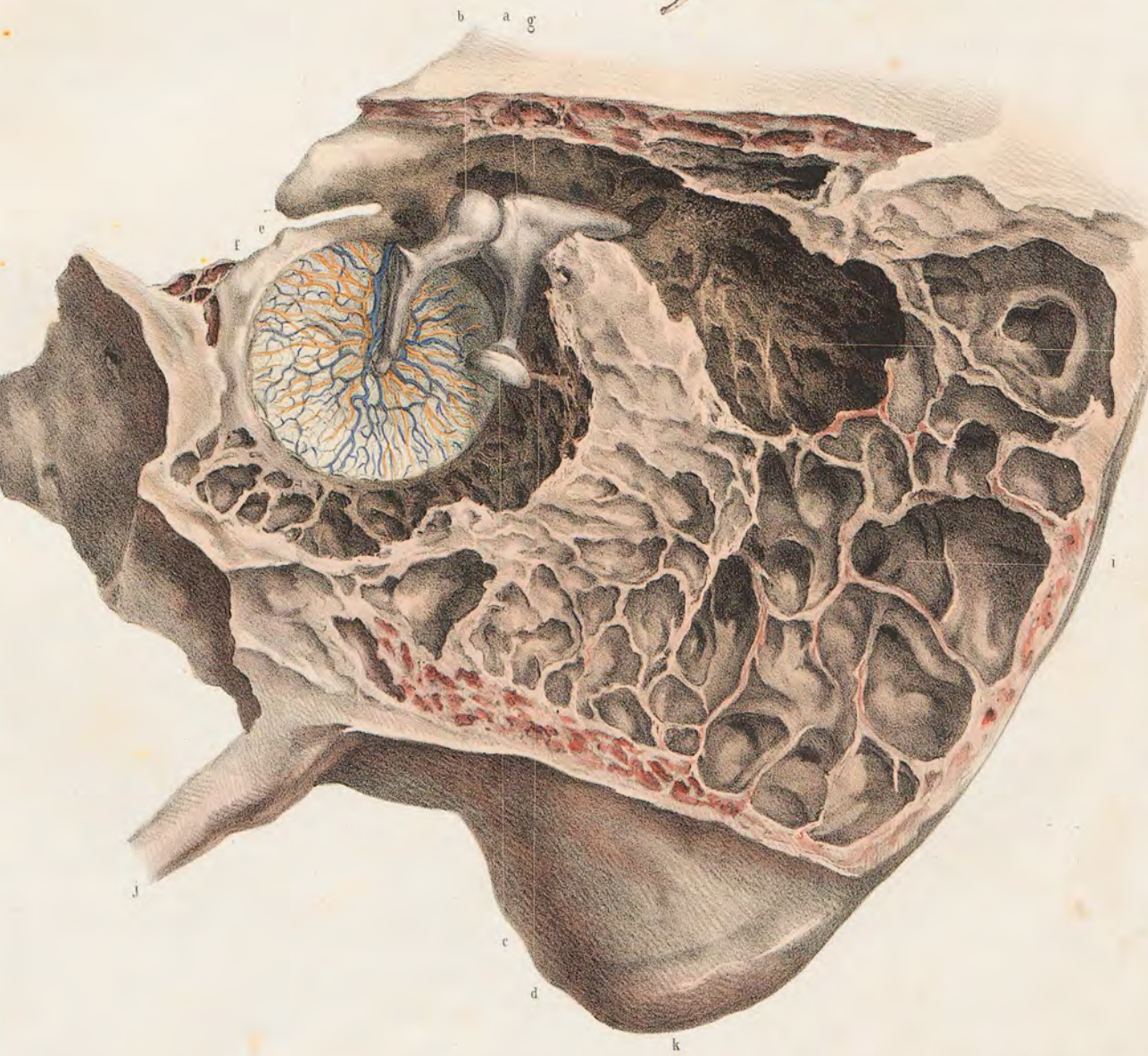


Fig. 5.



Préparation par Ludovic
Dessiné d'après nature par E. Pochet

Imp. Lemercier r. de Seine 57.

9 Summary and conclusion

Summary
Conclusion
Future perspectives

9.1 SUMMARY

Chapter 1 consists of an overview of current insights in the clinical characteristics, genetics and management of paraganglioma and pheochromocytoma patients.

In **chapter 2**, the clinical characteristics and treatment strategies of 147 patients with a total of 289 head and neck paragangliomas treated at the Amsterdam University Medical Centres, Vrije Universiteit Amsterdam, are evaluated. Variable clinical manifestations, such as multifocality (54%), associated sympathetic paraganglioma (1%), concurrent pheochromocytoma (3%), hypersecretion of catecholamines (30%) and/or metastatic disease (2%) were encountered. Sixty-five percent of DNA tested patients carried a pathogenic variant in *SDHD*, 10% in *SDHB* and 1% in *SDHAF2*. None of the *SDHB* variant carriers proved to harbour metastatic disease or developed a pheochromocytoma or sympathetic paraganglioma. Over a 60-year period a decreasing number of head and neck paragangliomas were surgically resected. Conversely, active surveillance has become a more prevalent treatment strategy and is now often the initial management option of choice. The growing understanding of the genetic predisposition, the associated clinical risk profiles (phenotype) of head and neck paraganglioma patient subgroups, and better understanding of the natural course has resulted in a more conservative management of head and neck paraganglioma patients.

In **chapter 3** a novel *SDHB* gene variant is associated with the formation of head and neck paraganglioma. In this family-based study, the DNA of 18 family members was tested, resulting in the identification of 10 carriers of an exon 1-3 deletion in the *SDHB* gene. One patient had a (presymptomatic) carotid body tumor, elevated catecholamine levels and high blood pressure, which normalized after surgical resection of the tumor. Negative *SDHB* immunostaining of the carotid body paraganglioma corroborated the hypothesis that it was caused by the *SDHB* variant. Thus, this deletion of exon 1-3 in the *SDHB* gene is a novel germline variant associated with the formation of hereditary paraganglioma.

In **chapter 4**, an extended family with a founder exon 3 deletion in the *SDHB* gene is studied. Seventeen variant carriers were identified, of whom 6 were clinically affected paraganglioma patients. The estimated penetrance for this *SDHB* exon 3 deletion at the ages of 40, 50, 60 and 70 was 4%, 9%, 15% and 21% respectively. The low 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 previous reports due to

various forms of bias. Previous penetrance calculations were prone to overestimation because of the limited inclusion of unaffected variant carriers and because the variant carriers are identified via index patients. This might result in a higher chance of selecting other variant carriers with the disease (ascertainment bias). In the current study, we included a relatively large number of unaffected variant carriers and corrected for ascertainment bias. This resulted in reduced estimates of *SDHB*-linked penetrance, an important finding for adequate (genetic) counseling of *SDHB*- variant carriers.

In **chapter 5**, the phenotypical characteristics of a nationwide cohort of *SDHB* variant carriers are determined and differences in clinical phenotypes related to specific *SDHB* variants were assessed. In a retrospective, descriptive study 194 *SDHB* variant carriers were included in seven clinical genetics centers. This cohort consisted of 83 (42.8%) disease-affected variant carriers and 111 (57.2%) unaffected variant carriers. Fifty-four carriers (27.8%) were clinically affected with one or more head and neck paragangliomas. Only four patients (2.1%) were clinically affected with a pheochromocytoma and 26 (13.4%) with one or more sympathetic paragangliomas. The ratios for pheochromocytoma and sympathetic paragangliomas found in our study were lower than previously reported in cohorts of *SDHB* variant carriers. The number of patients affected with at least one head and neck paraganglioma was relatively high (27.8%) compared with other studies (3-31%). By the inclusion of a large number of unaffected variant carriers through clinical genetics centers rather than through medical centers, the role of ascertainment bias was reduced. The cascade testing of relatives and structured follow-up protocols of *SDHB* variant carriers in the Netherlands identifies a relatively high number of asymptomatic variant carriers, with or without associated tumors, allowing for a more accurate representation of the phenotype of *SDHB* mutation carriers. Only fifteen patients (7.7%) developed metastatic disease and 17 patients (8.8%) developed non-paraganglionic tumors, including 5 renal cell carcinomas and 2 gastric gastrointestinal stromal tumors. Statistical analyses showed no significant differences between the carriers of the two most prevalent founder mutations in *SDHB* (exon 3 deletion and c.423+1G>A) for the number and location of head and neck paragangliomas, sympathetic paragangliomas or pheochromocytomas, nor in the occurrence of metastatic disease or non-paraganglionic tumors. This study underlines the importance of the inclusion of unaffected variant carriers in studies that assess the phenotypes of pathogenic germline variants. Including asymptomatic carriers provides a more accurate insight into the true spectrum of disease. The results from this study are important to consider in the clinical management and genetic counseling of families affected by *SDHB*-linked paraganglioma/pheochromocytoma syndromes.

Chapter 6 focuses on the clinical characteristics and outcome of treatment of 54 head and neck paraganglioma patients carrying a pathogenic variant in *SDHB* (selected from the nationwide cohort described in chapter 5). Only 8 patients (15%) had multiple paragangliomas, contrasting to what is known from *SDHD*-linked patients, who suffer from multiple paragangliomas much more frequently (in 60–79%). One patient (2%) harbored a concurrent pheochromocytoma and 3 (6%) developed metastatic disease. Twenty-seven patients (50%) had an operation for their head and neck paraganglioma and 15 (28%) received radiotherapy. Although the mean age at diagnosis in this cohort was relatively high (45.9 years), the youngest patient developed a head and neck paraganglioma at the age of 11 years, and an 18-year-old patient had already developed metastatic disease. In agreement with these findings, tumor screening in *SDHB*-linked families in the Netherlands nowadays starts at the age of 10 years. If an *SDHB* variant is identified in a head and neck paraganglioma patient, the clinician should be aware of the variable manifestations of the *SDHB*-linked tumor syndrome, the risk of hypersecretion of catecholamines, concurrent pheochromocytoma, and the association with non-paraganglionic tumors. Adequate surveillance of *SDHB* germline variant carriers is mandatory and should include screening for catecholamines or their metabolites, along with periodic radiological investigation of the abdomen, the pelvic region, thorax, and head and neck region.

In **chapter 7** the penetrance of paraganglioma and pheochromocytoma in *SDHB* variant carriers is calculated in the nationwide cohort, using a novel maximum likelihood estimator. This estimator addresses ascertainment bias and missing data on pedigree size and structure. A total of 195 *SDHB* variant carriers were included, carrying 27 different *SDHB* mutations. The 2 most prevalent *SDHB* genetic variants were known Dutch founder mutations: a deletion in exon 3 (31% of mutation carriers) and the c.423+1G>A mutation (24% of mutation carriers). One hundred and twelve carriers (57%) showed no physical, radiological or biochemical evidence of paraganglioma or pheochromocytoma. Fifty-four patients had a head and neck paraganglioma (28%), 4 patients had a pheochromocytoma (2%), 26 patients an sympathetic paraganglioma (13%). Using the novel estimator, the overall penetrance of *SDHB* variants is estimated to be 21% at age 50 and 42% at age 70. These estimates are lower than previously reported penetrance estimates of *SDHB*-linked cohorts, and confirm the penetrance estimates found in chapter 4. Similar disease risks are found for different *SDHB* germline variants as well as for male and female *SDHB* variant carriers.

In **chapter 8** the mortality of a nationwide cohort of *SDHB* variant carriers and that of a large cohort of *SDHD* variant carriers is estimated and compared to the

mortality of a matched cohort of the general Dutch population. A total of 192 *SDHB* variant carriers and 232 *SDHD* variant carriers were included in this study. The Standard Mortality Ratio (SMR) for *SDHB* mutation carriers is 1.89, increasing to 2.88 in carriers affected by paraganglioma and/or pheochromocytoma. For *SDHD* variant carriers the SMR is 0.93, and 1.06 in affected carriers. Compared to the general population, mortality seems to be increased in *SDHB* variant carriers, especially in those affected by paraganglioma and/or pheochromocytoma. In *SDHD* variant carriers, the mortality is comparable to that of the general Dutch population, even if they are affected by paraganglioma and/or pheochromocytoma. This finding is an poignant example of the differences in clinical risks conferred by different *SDHx* genes. It emphasizes the significance of DNA-testing in all paraganglioma and pheochromocytoma patients, and may warrant gene-specific management strategies.

9.2 CONCLUSION

Since the year 2000 we have witnessed an evolution in the care of patients with paraganglioma and pheochromocytoma. An expanding number of gene variants are now associated with paraganglioma and pheochromocytoma development, and the genetic heterogeneity in paragangliomas is unrivaled by any other human neoplasm. The different paraganglioma susceptibility genes all cause identical paragangliomas, but different clinical risks (phenotypes). A better understanding of the genotype-phenotype relationships increases the accuracy of clinical prediction, facilitates the design of optimal surveillance programs for asymptomatic carriers, and may help in tailor-made clinical decisions. The current thesis describes the clinical characteristics of *SDHB*-linked disease in two *SDHB*-linked families and in a Dutch nationwide cohort of *SDHB* variant carriers.

By studying a four-generation family, a novel *SDHB* gene variant could be associated with the formation of head and neck paraganglioma, a deletion of exon 1-3 in the *SDHB* gene.
In a second extended family associated with a known Dutch founder exon 3 deletion in the *SDHB* gene, we were able to calculate the penetrance for this specific gene variant. At the ages of respectively 40, 50, 60 and 70 the penetrance was 4%, 9%, 15% and 21%, much lower than previous estimates for *SDHB*-linked penetrance. This reduction in the estimated *SDHB*-linked penetrance was facilitated by the inclusion of a relatively large number of unaffected variant carriers. The study of a nationwide Dutch cohort of *SDHB* variant carriers revealed that they have an intermediate risk for the development of head and neck paragangliomas

(27.8%), a low risk for the development of pheochromocytomas (2.1%), an intermediate risk for the development of sympathetic paragangliomas (13.4%) and a relatively low risk of developing metastatic disease. By using a novel maximum likelihood method for penetrance calculations, the overall penetrance of *SDHB* variants is estimated to be 21% at age 50 and 42% at age 70, confirming that *SDHB*-linked penetrance is lower than previously reported. Similar disease risks were found for different *SDHB* variants as well as for male and female *SDHB* variant carriers.

Compared to a matched cohort of the general population, mortality is increased in *SDHB* variant carriers but not in *SDHD* variant carriers. There is an interesting paradox behind this observation, illustrating the differences in clinical risks between carriers of pathogenic *SDHB* and *SDHD* variants. Whereas *SDHD*-linked carriers will develop one or more (head and neck) paragangliomas in most cases (88-100%), mortality for the group as a whole is not increased. Conversely, only a minority of *SDHB*-linked carriers will develop a paraganglioma and/or pheochromocytoma (43%), but this does result in a higher mortality rate – an effect that seems to be attributable to the clinically affected *SDHB*-linked carriers.

In summary, this thesis increases our understanding of *SDHB* gene variants and the associated clinical picture in The Netherlands, and reveals new insights especially in the penetrance and mortality of *SDHB*-linked disease.

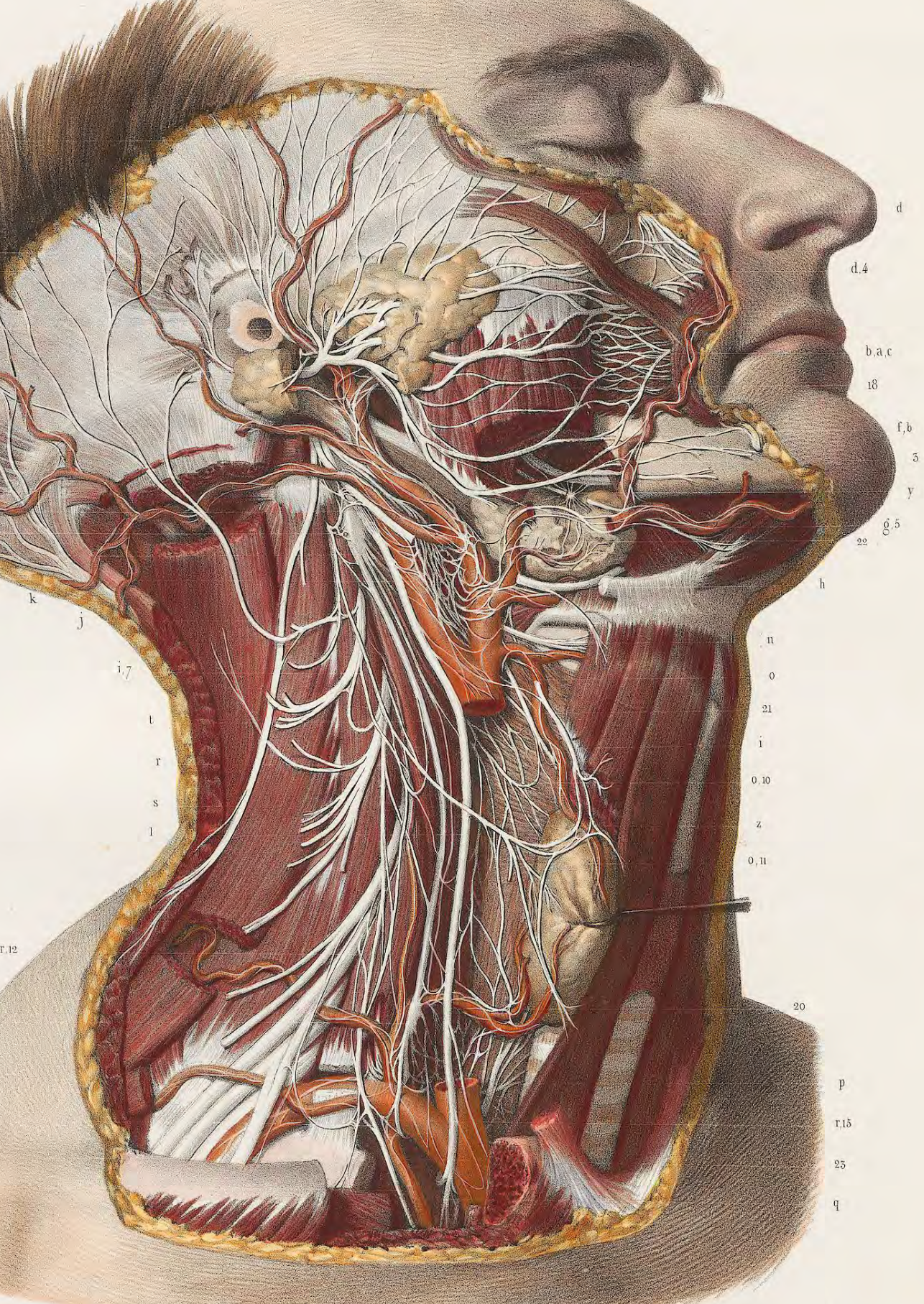
9.3 FUTURE PERSPECTIVES

It is intriguing that the causative gene determines variation in the phenotype and prognosis for paraganglioma and pheochromocytoma patients, even though pathogenic variants in different *SDH* genes cause paraganglioma syndrome through defects in the same protein complex (*SDH*). What is more, there may be evidence that this phenotypic divergence is not just dependent on the gene but also on variant class (i.e. missense/nonsense). The further dissection and refinement of variant-specific phenotypes will possibly shed more light on the ways in which the disruption of the gene causes a paraganglioma, and likely further our understanding of genotype-phenotype correlations, leading to more personalized risk assessment, counseling, and therapies. A better understanding of the tumor biology might also increase the available treatment options for paragangliomas and pheochromocytomas, especially for malignant and multiple paragangliomas. As of yet, there are no curative options for metastasized disease. Research is needed to evaluate therapies with novel

mechanisms of action. The use of tyrosine kinase inhibitors, radionuclide agents, and targeted immunotherapy may improve the outcomes of patients with malignant or multiple paragangliomas and pheochromocytomas in the future.

Pathogenic *SDHB* variants seem to confer higher risks to patients as opposed to, for instance, *SDHD* variant carriers. This is reflected by the higher mortality ratio for carriers of pathogenic *SDHB* variants reported in this thesis. Based on this finding, a more aggressive treatment strategy may be warranted in *SDHB*-linked paraganglioma patients. However, the effect of early and/or more aggressive intervention on malignant transformation or mortality rates in *SDHB*-linked patients is still unknown, and current treatment options such as surgery and radiotherapy come with inherent risks to the patient. Furthermore, the clinical course of the disease varies widely within the group *SDHB*-linked patients, an thus not all will benefit equally from such a shift in management strategy. Future studies will hopefully help to distinguish those patients that would benefit from specific interventions from those who don't, thereby improving the outcome for paraganglioma patients without exposing them unnecessarily to therapy-related risks.

In the past, paraganglioma research has been a wonderful example of how the study of a rare condition can elucidate basic principles in biological and pathogenic processes and facilitate discoveries that are applicable in a much broader context. It has played a role in the recognition and understanding of the role of the metabolism in tumorigenesis, shed light on the mechanisms behind peculiar modes of inheritance, and has shown the genetic heterogeneity that may underlie a specific disease. In this thesis too, a novel method for penetrance calculations was developed to meet the challenges set by *SDHB*-linked paraganglioma syndrome, and as a result a more robust methodology for penetrance calculations is now available that is useful not just for paraganglioma but for penetrance estimations in general, especially in rare and low-penetrant hereditary disease, when data on pedigree size and structure are missing. Hopefully future paraganglioma research will not only provide new insights in paraganglioma pathogenesis, clinical characteristics and treatment, but also continue to contribute to the wider field of medicine.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f,b
5
y
g,5
22
h
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j
i,7
t
r
s
l
r,12
n
o
21
i
o,10
z
o,11
20
p
r,15
23
q

10 Addendum

Samenvatting en conclusie
List of publications
Curriculum vitae
Dankwoord

10.1 SAMENVATTING EN CONCLUSIE

Samenvatting

Hoofdstuk 1 bestaat uit een overzicht van de huidige inzichten met betrekking tot de klinische kenmerken, genetica, diagnostiek en behandelingsstrategieën van patiënten met een paraganglioom en feochromocytoom.

In **hoofdstuk 2** worden de klinische kenmerken en behandelingsstrategieën van 147 hoofd-halsparaganglioom patiënten - met in totaal 289 tumoren – geëvalueerd. Verschillende klinische manifestaties zijn waargenomen, zoals multifocaliteit (54%), een synchroon sympathisch paraganglioom (1%) of feochromocytoom (3%), hypersecretie van catecholamines (30%) of gemetastaseerde ziekte (2%). Van de patiënten die een DNA-test hebben ondergaan is 65% drager van een mutatie in *SDHD*, 10% van een mutatie in *SDHB* en 1% van een mutatie in *SDHAF2*. Geen van de *SDHB*-mutatiedragers heeft een feochromocytoom, sympathisch paraganglioom of gemetastaseerde ziekte. In de afgelopen 60 jaar is het percentage hoofd-halsparagangliomen dat operatief werd verwijderd aanzienlijk gedaald. Steeds vaker werd gekozen voor een '*active surveillance*' behandelingsstrategie. Meer kennis van (I) de genetische predispositie, (II) de geassocieerde klinische risicoprofielen (fenotype), en (III) het natuurlijke beloop van deze tumoren heeft geresulteerd in een meer conservatieve aanpak van patiënten met een hoofd-halsparaganglioom.

In **hoofdstuk 3** wordt een nieuwe *SDHB*-mutatie beschreven die samenhangt met de ontwikkeling van een hoofd-halsparaganglioom. In deze studie is het DNA van 18 leden van één familie getest. Gebleken is dat 10 leden drager zijn van een exon 1-3 deletie in het *SDHB*-gen. Eén familielid had een (presymptomatisch) paraganglioom ter plaatse van de carotisbifurcatie, een hoge bloeddruk en verhoogde catecholamine waarden, die beiden normaliseerden na operatieve verwijdering van de tumor. Een negatieve *SDHB*-immunokleuring op het tumorweefsel heeft bevestigd dat deze tumor wordt veroorzaakt door de *SDHB*-mutatie. Deze niet eerder beschreven deletie van exon 1-3 in het *SDHB*-gen is een nieuwe erfelijke variant, die geassocieerd wordt met de ontwikkeling van paragangliomen.

In **hoofdstuk 4** wordt een grote familie beschreven met een exon 3 deletie in het *SDHB*-gen. Zes van de 17 geïdentificeerde mutatiedragers hebben een paraganglioom. De geschatte penetrantie van deze mutatie op de leeftijd van 40, 50, 60 en 70 jaar is respectievelijk 4%, 9%, 15% en 21%. Deze lage penetrantie zou een klinisch kenmerk van deze exon 3 deletie kunnen zijn. Een andere mogelijkheid is

dat deze wordt veroorzaakt door een gedeelde genetische- of omgevingsfactor binnen deze familie, die van invloed is op de penetrantie. Eerdere studies beschrijven een hogere penetrantie van *SDHB*-mutaties, vermoedelijk ten gevolge van verschillende vormen van bias. Deze eerdere berekeningen van penetrantie zijn vatbaar voor overschatting vanwege (I) de beperkte inclusie van niet-aangedane mutatiedragers en (II) de identificatie van mutatiedragers via indexpatiënten. Dit resulteert in een verhoogde kans op de selectie van aangedane mutatiedragers (ascertainment bias). In de huidige studie hebben we een relatief groot aantal niet-aangedane mutatiedragers geïncludeerd en is gecorrigeerd voor ascertainment bias. Voorgaande heeft geleid tot een gereduceerde schatting van *SDHB*-geassocieerde penetrantie, hetgeen belangrijk is voor een adequate (genetische) counseling van *SDHB*-mutatiedragers.

In **hoofdstuk 5** komen de klinische kenmerken van *SDHB*-mutatiedragers in een landelijk cohort aan de orde. In deze retrospectieve, beschrijvende studie zijn 194 *SDHB*-mutatiedragers geïncludeerd vanuit zeven klinisch-genetische centra. Het cohort bestaat uit 83 (42.8%) aangedane mutatiedragers en 111 (57.2%) niet-aangedane mutatiedragers. Vierenvijftig mutatiedragers (27.8%) hebben één of meer hoofd-halsparagangliomen. Slechts 4 patiënten (2.1%) hebben een feochromocytoom en 26 (13.4%) één of meer sympathische paragangliomen; een aanzienlijk lager risico dan staat beschreven in eerdere studies over *SDHB*-mutatiedragers. Het aantal patiënten met ten minste één hoofd-halsparaganglioom is relatief hoog (27.8%) in vergelijking met andere studies (3-31%). Omdat in de onderhavige studie een groot aantal niet-aangedane mutatiedragers is geïncludeerd, blijft de ascertainment bias beperkt. In Nederland wordt aan familieleden van *SDHB*-mutatiedragers structureel genetisch onderzoek aangeboden. Indien een familielid drager blijkt te zijn van de mutatie wordt er volgens een vast protocol vervolgonderzoek aangeboden. Hierdoor wordt een relatief groot aantal asymptomatische mutatiedragers - met of zonder geassocieerde tumoren – geïdentificeerd. Deze aanpak zorgt voor een nauwkeurige weergave van het fenotype van *SDHB*-mutatiedragers. Slechts 15 patiënten (7.7%) hebben gemetastaseerde ziekte en 17 patiënten (8.8%) een niet-paraganglion gerelateerde tumor, waaronder 5 niercelcarcinomen en 2 gastro-intestinale stromale tumoren. Statistische analyses tonen geen significante verschillen tussen de dragers van de twee meest voorkomende mutaties in *SDHB* (exon 3 deletie en c.423 + 1G> A) betreffende het aantal en de locatie van hoofd-halsparagangliomen, sympathische paragangliomen of feochromocytomen. Er zijn ook geen significante verschillen zichtbaar in het voorkomen van gemetastaseerde ziekte of de aanwezigheid van niet-paraganglion gerelateerde tumoren voor deze mutaties. In dit hoofdstuk wordt het belang onderstreept van het includeren van niet-aan-

gedane mutatiedragers in studies waar het fenotype van een pathogene mutatie wordt beoordeeld. De inclusie van asymptomatische mutatiedragers geeft een beter zicht op het ware spectrum van de ziekte. De beschreven resultaten zijn van belang voor de genetische counseling en behandeling van families, die zijn aangedaan door *SDHB*-geassocieerde ziekte.

Hoofdstuk 6 richt zich op de klinische kenmerken en behandelingsstrategieën van 54 dragers van een pathogene mutatie in *SDHB* met een hoofd-halsparaganglioom (geselecteerd uit het landelijke cohort beschreven in hoofdstuk 5). Slechts 8 patiënten (15%) hebben meerdere paragangliomen, in tegenstelling tot *SDHD* mutatiedragers, die vaak meerdere paragangliomen hebben (60-79%). Eén patiënt (2%) heeft tevens een feochromocytoom en 3 patiënten (6%) hebben gemetastaseerde ziekte. Zevenentwintig patiënten (50%) zijn geopereerd vanwege het hoofd-halsparaganglioom en 15 patiënten (28%) zijn radiotherapeutisch behandeld. Hoewel de leeftijd waarop de diagnose hoofd-halsparaganglioom in dit cohort gesteld werd relatief hoog is (45.9 jaar), had de jongste patiënt op 11-jarige leeftijd een hoofd-halsparaganglioom en een 18-jarige patiënt bleek reeds gemetastaseerde ziekte te hebben. Overeenkomstig deze onderzoeksresultaten start screening op paragangliomen in *SDHB*-geassocieerde families tegenwoordig op de leeftijd van 10 jaar in Nederland. Indien een patiënt met een hoofd-halsparaganglioom drager is van een *SHDB*-mutatie moet de behandelend arts op de hoogte zijn van de verschillende manifestaties die het *SDHB*-geassocieerde tumorsyndroom met zich mee kan brengen: hypersecretie van catecholamines, aanwezigheid van een sympathisch paraganglioom of feochromocytoom en de associatie met niet-paraganglion gerelateerde tumoren. Adequate surveillance van *SDHB*-mutatiedragers wordt sterk aanbevolen en omvat screening op catecholamines of hun metabolieten, in combinatie met periodiek radiologisch onderzoek van de buik, bekkenregio, thorax en het hoofd-halsgebied.

In **hoofdstuk 7** wordt de penetrantie berekend in het landelijke cohort *SDHB*-mutatiedragers (zie hoofdstuk 5) middels een nieuwe maximum-likelihood-schatter. Deze schatter houdt rekening met ontbrekende gegevens over stamboomgrootte en –structuur en ascertainment bias. In totaal zijn 195 *SDHB*-mutatiedragers geïncludeerd, die 27 verschillende *SDHB*-mutaties dragen. De twee meest voorkomende *SDHB*-mutaties zijn bekende Nederlandse foundermutaties: een exon 3 deletie (31% van de mutatiedragers) en een c.423 + 1G>A mutatie (24% van de mutatiedragers). Bij 112 dragers (57%) zijn er geen aanwijzingen voor de aanwezigheid van een paraganglioom of feochromocytoom bij lichamelijk, radiologisch of biochemisch onderzoek. Vierenvijftig patiënten hebben een hoofd-halspara-

ganglioom (28%), 4 patiënten een feochromocytoom (2%) en 26 patiënten een sympathisch paraganglioom (13%). Met behulp van de nieuwe schatter wordt de penetrantie van *SDHB*-mutatiedragers geschat op 21% op de leeftijd van 50 jaar en 42% op de leeftijd van 70 jaar. Deze schatting ligt lager dan eerder gerapporteerde penetrantieschattingen van *SDHB*-gerelateerde cohorten en bevestigt de schatting van penetrantie van *SDHB* in hoofdstuk 4. Soortgelijke ziekterisico's worden gevonden bij verschillende *SDHB*-mutaties en voor mannelijke en vrouwelijke *SDHB*-mutatiedragers.

De mortaliteit van een landelijk cohort *SDHB*-mutatiedragers en die van een groot cohort *SDHD*-mutatiedragers worden geschat in **hoofdstuk 8** en vergeleken met de mortaliteit van een gematcht cohort van de doorsnee Nederlandse bevolking. In totaal zijn 192 *SDHB*-mutatiedragers en 232 *SDHD*-mutatiedragers geïncludeerd in deze studie. De standaard mortaliteitsratio (SMR) van *SDHB*-mutatiedragers is 1.89, oplopend tot 2.88 van mutatiedragers met een paraganglioom of feochromocytoom. De SMR van *SDHD*-mutatiedragers is 0.93 en van aangedane mutatiedragers 1.06. Vergeleken met de doorsnee bevolking lijkt de mortaliteit verhoogd te zijn van *SDHB*-mutatiedragers, met name van diegenen met een paraganglioom of feochromocytoom. Van *SDHD*-mutatiedragers is de mortaliteit vergelijkbaar met die van de doorsnee Nederlandse bevolking, zelfs als de mutatiedrager een paraganglioom of feochromocytoom heeft. Dit resultaat is een saillant voorbeeld van het verschil in klinisch risico dat wordt veroorzaakt door verschillende *SDHx*-genen. Hierdoor wordt het belang van DNA-onderzoek voor alle patiënten met een paraganglioom of feochromocytoom onderschreven en deze bevindingen kunnen een gen-specifieke behandelingsstrategie rechtvaardigen.

Conclusie

De zorg voor patiënten met een paraganglioom of feochromocytoom heeft sinds het jaar 2000 een grote ontwikkeling doorgemaakt. Tegenwoordig is een groeiend aantal genmutaties geassocieerd met de ontwikkeling van deze tumoren en deze genetische heterogeniteit is ongeëvenaard door enig ander menselijk neoplasma. Mutaties in verschillende genen veroorzaken identieke paragangliomen, echter deze brengen een verschillend klinische risico (fenotype) met zich mee. Meer kennis van de genotype-fenotype-relatie verbetert de nauwkeurigheid van klinische voorspellingen, vergemakkelijkt het maken van optimale surveillance programma's voor asymptomatische mutatiedragers en is behulpzaam bij het maken van op maat gemaakte klinische beslissingen. Het onderhavige proefschrift beschrijft de klinische kenmerken van *SDHB*-geassocieerde ziekte in twee *SDHB*-geassocieerde families en in een landelijk Nederlands cohort van *SDHB*-mutatiedragers.

In het onderzoek van een familie van vier generaties is een nieuwe *SDHB*-mutatie (een exon 1-3 deletie in het *SDHB*-gen) geassocieerd met de ontwikkeling van een hoofd-halsparaganglioom. In een andere, grote familie met dragers van een bekende Nederlandse foundermutatie (een exon 3 deletie in het *SDHB*-gen) is de penetrantie berekend. Op de leeftijd van respectievelijk 40, 50, 60 en 70 jaar is de penetrantie 4%, 9%, 15% en 21% van deze specifieke mutatie. Deze schatting ligt aanzienlijk lager dan eerdere schattingen van *SDHB*-geassocieerde penetrantie. De bijgestelde schatting van penetrantie is het gevolg van de inclusie van een relatief groot aantal niet-aangedane mutatiedragers in de berekening. In een landelijke studie wordt gesteld dat *SDHB*-mutatiedragers een gemiddeld risico hebben op de ontwikkeling van een hoofd-halsparaganglioom (28%), een laag risico op de ontwikkeling van een feochromocytoom (2%), een gemiddeld risico op de ontwikkeling van een sympathische paraganglioom (13%) en een relatief laag risico op het ontwikkelen van gemetastaseerde ziekte (8%). Door een nieuwe maximum-likelihood-schatter te gebruiken voor de berekening van penetrantie wordt de penetrantie van *SDHB*-mutaties geschat op 21% op 50-jarige leeftijd en 42% op 70-jarige leeftijd, hetgeen bevestigt dat de *SDHB*-geassocieerde penetrantie lager ligt dan eerder is beschreven. Soortgelijke ziekterisico's zijn gevonden voor verschillende typen *SDHB*-mutaties evenals voor mannelijke of vrouwelijke *SDHB*-mutatiedragers.

Vergeleken met een gematcht cohort van de doorsnee bevolking is de mortaliteit van *SDHB*-mutatiedragers verhoogd, maar dit is niet het geval bij *SDHD*-mutatiedragers. Er schuilt een interessante paradox achter dit onderzoeksresultaat, die het verschil in klinisch risico tussen dragers van pathogene *SDHB*- en *SDHD*-mutaties illustreert. Terwijl *SDHD*-mutatiesdragers in de meeste gevallen (88-100%) één of meer (hoofd-hals)paragangliomen ontwikkelen, neemt de mortaliteit van de groep als geheel niet toe. Daarentegen zal slechts een minderheid van *SDHB*-mutatiedragers een paraganglioom of feochromocytoom ontwikkelen (43%), maar is de mortaliteit verhoogd - een effect dat toegeschreven lijkt te kunnen worden aan de klinisch aangedane *SDHB*-mutatiedragers.

Samenvattend vergroot dit proefschrift onze kennis van *SDHB*-mutaties en het geassocieerde klinische beeld in Nederland. Ook worden nieuwe inzichten onthuld, met name in de penetrantie en mortaliteit van *SDHB*-geassocieerde ziekte.

Toekomstperspectieven

Het is intrigerend dat het oorzakelijke gen bepalend is voor een variatie in fenotype en prognose van patiënten met een paraganglioom of feochromocytoom,

ondanks het feit dat mutaties in verschillende *SDH*-genen een paraganglioom-syndroom kunnen veroorzaken door defecten in hetzelfde eiwitcomplex (*SDH*). Bovendien lijkt er bewijs te zijn dat deze divergentie in fenotype niet alleen afhankelijk is van het gen, maar ook van het type mutatie (oftewel missense/nonsense). De verdere ontleding en verfijning van mutatie-specifieke fenotypes zal mogelijk meer duidelijkheid gaan geven over de manier waarop de verstoring van het gen een paraganglioom veroorzaakt. Deze aanpak zal ons begrip over genotype-fenotype-correlaties aannemelijk bevorderen, hetgeen tot een meer gepersonaliseerde risicoschatting, counseling en therapie zal leiden.

Een beter inzicht in de tumorbiologie kan ook de beschikbare behandelingsstrategieën voor patiënten met paragangliomen en feochromocytomen doen toenemen, met name voor patiënten met meervoudige of gemetastaseerde paragangliomen. Tot nu toe zijn er geen curatieve behandelopties voor patiënten met gemetastaseerde ziekte. Verder onderzoek is nodig om therapieën met nieuwe werkingsmechanismen te evalueren. Het gebruik van tyrosinekinaseremmers, radionucliden en gerichte immunotherapie zou het behandelresultaat van patiënten met meervoudige of gemetastaseerde paragangliomen in de toekomst kunnen verbeteren.

Pathogene *SDHB*-mutaties lijken grotere risico's voor patiënten met zich mee te brengen, in tegenstelling tot bijvoorbeeld *SDHD*-mutaties. Dit wordt weerspiegeld in de verhoogde mortaliteit van *SDHB*-mutatiedragers zoals wordt beschreven in dit proefschrift. Deze bevinding zou een meer agressieve behandelingsstrategie voor patiënten met een *SDHB*-mutatie rechtvaardigen. Het effect van een vroege of agressievere interventie op kwaadaardige transformatie of mortaliteit is echter nog onbekend voor *SDHB*-geassocieerde patiënten. De huidige behandelingsstrategieën, zoals chirurgie en radiotherapie, brengen inherente risico's met zich mee. Bovendien varieert het klinische beloop sterk binnen de groep patiënten met een *SDHB*-mutatie, waardoor mogelijk niet alle patiënten zullen profiteren van een dergelijke verschuiving van de behandelingsstrategie. Hopelijk zal verder onderzoek behulpzaam zijn in het differentiëren van patiënten met of zonder baat bij specifieke interventies. Op deze manier kan het resultaat van de behandeling bij patiënten met een paraganglioom worden verbeterd, zonder ze onnodig bloot te stellen aan therapie-gerelateerde risico's.

In het verleden is het paragangliomen onderzoek een prachtig voorbeeld gebleken van hoe het bestuderen van een zeldzame conditie opheldering kan verschaffen in basale, algemeen geldende biologische en pathogene mechanismen. Op deze manier zijn ontdekkingen gedaan die in een bredere context toegepast kun-

nen worden. Het onderzoek draagt bij aan een beter begrip en herkenning van het metabolisme in tumorgenese. Ook worden hierin de eigenaardige wijzen van overerving en de genetische heterogeniteit aangetoond, die aan een specifieke ziekte ten grondslag kunnen liggen. Uitgedaagd door de vragen bij het *SDHB*-geassocieerde paraganglioomsyndroom is in dit proefschrift een nieuwe methode ontwikkeld voor het berekenen van penetrantie. Hierdoor is een duidelijke methodologie beschikbaar voor penetrantieberekeningen, die niet alleen geschikt is voor berekeningen van paragangliomen, maar ook voor penetrantieberekeningen in het algemeen. Dat is vooral nuttig bij zeldzame en laag-penetrante erfelijke ziektes wanneer gegevens over stamboomgrootte en -structuur ontbreken. Hopelijk zal toekomstig paragangliomen onderzoek niet alleen nieuwe inzichten geven in de pathogenese, klinische kenmerken en behandeling van paragangliomen, maar ook de geneeskunde in de volle breedte blijven verrijken.

10.2 LIST OF PUBLICATIONS

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10.3 CURRICULUM VITAE

Johannes Rijken werd geboren op 8 januari 1986 te Veenendaal. In 2004 behaalde hij het eindexamen Atheneum aan het Ichthus College te Veenendaal. In datzelfde jaar werd begonnen met de studie geneeskunde aan de Erasmus Universiteit in Rotterdam. Tijdens zijn studie deed hij onderzoek naar cerebrale malaria in Chittagong, Bangladesh, met een onderzoeksteam uit Bangkok en Oxford. In 2010 rondde hij de studie geneeskunde cum laude af en startte als assistent heelkunde in het Ijsselland ziekenhuis te Capelle aan den Ijssel. Hier werd zijn enthousiasme over de heelkunde bevestigd, vooral over de heelkunde van het hoofd-halsgebied. Onder leiding van prof.dr. C.R. Leemans begon hij in 2011 met de specialisatie Keel-, Neus-, en Oorheelkunde (KNO) in het VU medisch centrum te Amsterdam. Een gedeelte van de opleiding werd in het Tergooi Ziekenhuis gevolgd in Blaricum onder leiding van dr. J. Borgstein en in het Spaarne Gasthuis in Hoofddorp onder leiding van dr. E.J. van Nieuwkerk. Onder begeleiding van dr. E.F. Hensen en prof. dr. C.R. Leemans werd eind 2014 begonnen met het verrichten van onderzoek naar patiënten met een paraganglioom, hetgeen de basis vormde voor dit proefschrift. Tijdens zijn opleiding reisde hij driemaal af naar Mumias, Kenia, namens de stichting Eardrop, om clinical officers te trainen in de KNO en hoofd-halschirurgie. In 2016 werd hij door de Memory Group verkozen tot de top 100 best young professionals van Nederland. Nadat de opleiding in september 2016 was afgerond startte hij met een tweejarig fellowship hoofd-halschirurgie in het VU medisch centrum. Tijdens deze twee jaar volgde hij met succes het internationale online fellowship hoofd-halschirurgie en oncologie, onder leiding van prof. J.P. Shah. Na het afronden van het fellowship werkte hij als staflid in het VU medisch centrum te Amsterdam. In juni 2019 is hij gestart als KNO-arts/ hoofd-halschirurg op de afdeling Hoofd-Hals Chirurgische Oncologie van het Universitair Medisch Centrum Utrecht (afdelingshoofd prof.dr. R. de Bree). Johannes is getrouwd met Anne, en is vader van Jan (2016) en Huib (2018).

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De leden van de promotiecommissie, prof.dr. W. Wisselink, prof.dr. P. Devilee, prof. dr. M. den Heijer, prof.dr. R.J. Baatenburg de Jong, prof.dr. B. Kremer en prof.dr. R.J. Stokroos wil ik bedanken voor de nauwgezette beoordeling van dit manuscript. Prof.dr. J.C. Jansen, beste Jeroen, dank dat je deel wilt nemen aan de zitting en voor je waardevolle suggesties tijdens het onderzoek, vaak vanuit vogelperspectief.

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