

**Pheochromocytoma, paraganglioma, glomus tumors, and associated syndromes:  
multiple endocrine neoplasia type 2, von Hippel-Lindau syndrome,  
neurofibromatosis type 1, and paraganglioma syndrome type 1-4**

**Informations for patients and their families**

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## **PREFACE AND ACKNOWLEDGEMENT**

This brochure is intended to give thorough information on pheochromocytoma and glomus tumors (paragangliomas) and their assignment to inherited forms. It originated from the expressed wishes of our patients and on the background of years of clinical and scientific work and multiple scientific publications covering this complex topic. This brochure is based on the results of collaborations with many colleagues in Freiburg, in Germany, and abroad. I would like to use this opportunity to thank them for the countless encounters, personal or by e-mails, that were either related to a specific family history or a scientific project. For the original German version I would like to thank my laboratory in Freiburg and many colleagues in other specialties in Freiburg that are mentioned on special page for proofreading the manuscript and for many ideas. Some results from scientific publications that were coordinated by me or that I was part of have been used for this brochure. They are listed in the literature section of this brochure.

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## **1. TIPS TO THE READERS**

This brochure on pheochromocytoma, paraganglioma, glomus tumors, and other associated syndromes is intended for patients and provides the most recent information based on the highest standards of care.

It is important to recognize the many different perspectives of the readers of the information provided in this patient guide. For example, the patient may be at the stage where a tumor may be a consideration among others as a cause of signs and symptoms, or the tumor may have been diagnosed but not yet removed, or the tumor may have been removed and the patient is in the process of long term follow-up. Other readers may be seeking information on the role for genetic testing or the clinical significance of specific mutation test results. Although we could have written a separate brochure for each of these topics, that approach would have resulted in a large amount of overlapping and repetitious information. Thus, we endeavored to provide that reader with a broad but concise patient guide for pheochromocytoma, paraganglioma, glomus tumors, and other associated syndromes. Each section includes commentary on the key questions and answers.

This brochure is based on years of experience in our special clinic for these patients and the scientific research in clinical and molecular genetic aspects of these diseases in Freiburg, Germany. Many figures are included to illustrate key imaging examples and concepts. We tried our best to use lay language for all explanations. We are very grateful for suggestions to improve this brochure and will incorporate these suggestions in future up-dated versions of this brochure.

## **2. CRITERIA OF QUALITY FOR CENTERS FOR PHEOCHROMOCYTOMA AND GLOMUS TUMORS**

Patients with pheochromocytoma and paraganglioma should be treated in medical centers with special experience in this area. It is necessary but not sufficient that the knowledge presented in this brochure exists in such a center. Considerable practical experience is also necessary. Since this disease is rare, the number of newly diagnosed patients per year is not very high. A minimum of 10 patients with pheochromocytoma per year should be diagnosed. Even some large medical centers will not reach this number which is concerning for the patients. Taking into consideration that different physicians diagnose and operate on the patients, it is understandable that some patients have unsatisfying experience. The integrative preventive medical examinations should include molecular diagnostic and consultations. These modern methods of analysis require specialized laboratories, genetic consultation, and clinical support as part of preventive medicine. Patients will certainly welcome that specialized centers treat them according to these guidelines and will even willingly accept long commutes to these centers. Therefore, the adequate treatment of patients with pheochromocytoma in integrated interdisciplinary medical centers is recommended and should be the standard of care for the future.

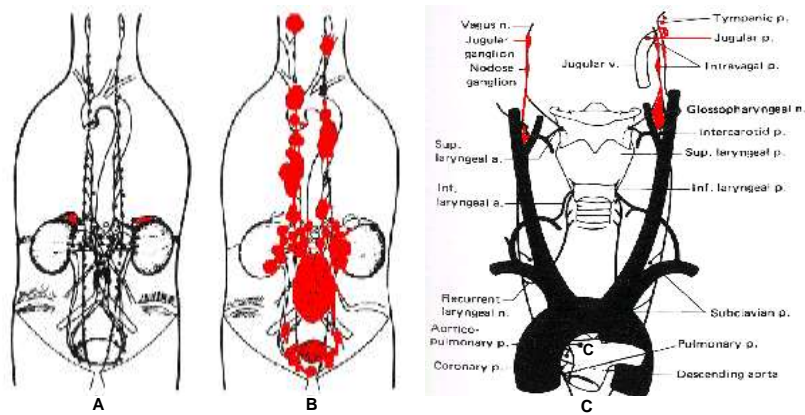
### 3. WHAT IS A PHEOCHROMOCYTOMA? WHAT IS A GLOMUS TUMOR?

The nervous system regulates a variety of processes in the human body. Many of these processes are autonomically (ie, “automatically”) regulated; for instance heart rate, blood pressure, blood oxygen levels, blood base/acid (pH) regulation, respiration, blood supply to organs, temperature regulation of the body, and digestion. The human body has a special, wide regulatory network called the autonomic or paraganglial nervous system, with paraganglia (Figure 1). The adrenal glands, specifically the core of the adrenal glands (called “medulla”), are the largest paraganglia in the body. The adrenal glands are approx. 3 x 3 x 1 cm in size and are located above the kidneys. The adrenal gland consists of a core (“medulla”) and a surrounding cortex. Tumors derived from the adrenal medulla are called pheochromocytomas (Figures 1, 2).

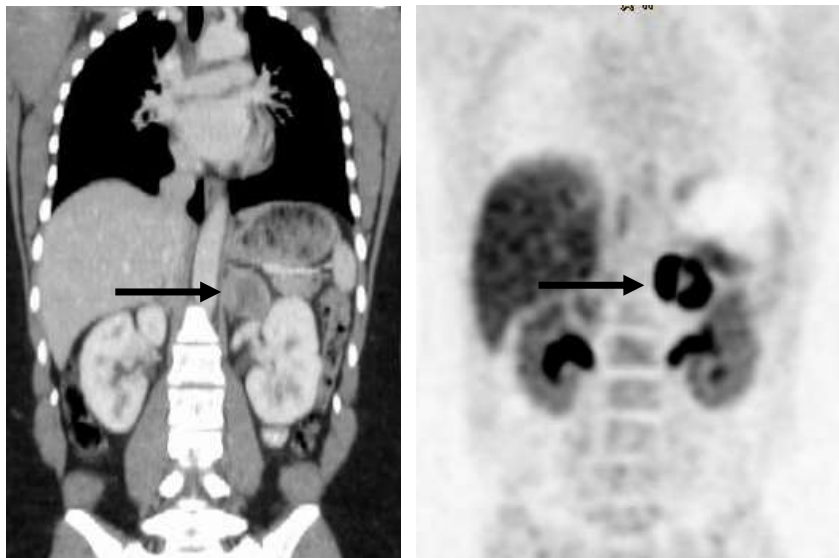
Paraganglia are located in many different locations in the body, in particular in the chest and the abdomen, frequently in close proximity of large arteries. Tumors that develop in these paraganglia are called extra-adrenal pheochromocytoma (located outside the adrenal glands) (Figure 3).

**Pheochromocytomas** (Figure 2 to 4) are mostly benign and do not metastasize. Pheochromocytomas produce excess amounts of adrenaline (or epinephrine) and noradrenaline (or norepinephrine), both hormones are produced by the adrenal glands and other paraganglia and are important for their function. These hormones and their metabolites metanephrine, normethanephrine, and vanillylmandelic acid can be detected in the blood and urine. The symptoms of pheochromocytoma are largely due

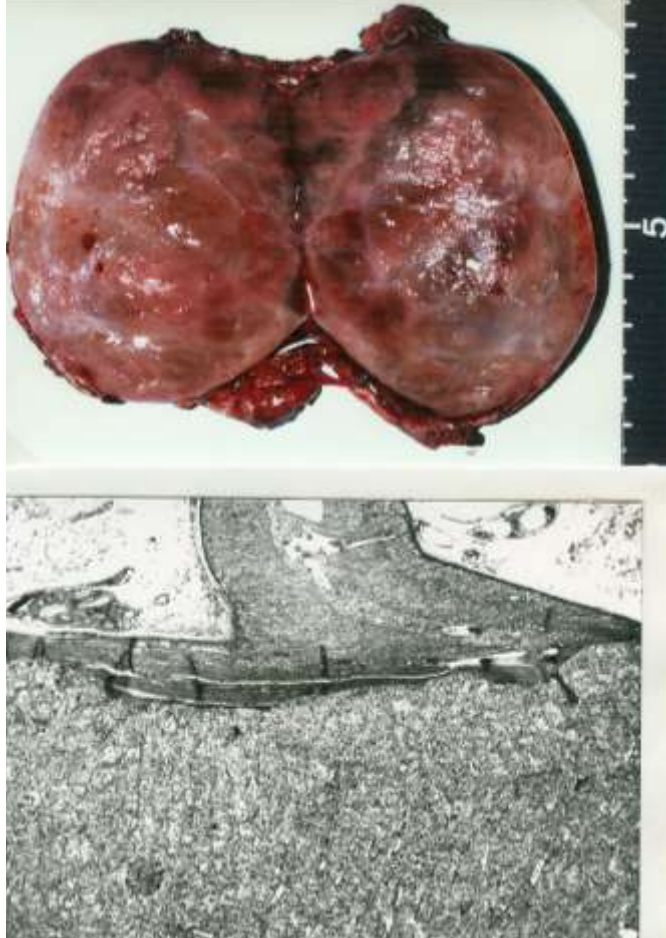




**Figure 1:** The paraganglial system and locations of pheochromocytoma in the adrenal glands (left), extra-adrenal pheochromocytoma (middle), and glomus tumors (right). The locations of pheochromocytoma and glomus tumors are depicted in red. A, B from Manger and Gifford, *J Clin Hypertens* 2002; 4:62-72 with permission of Dr Manger, C from Glenner CC, Grimley PM *Tumors of the extra-adrenal paraganglion system*, Atlas of Tumor Pathology, 2<sup>nd</sup> series, fascicle 9, Washington DC, AFIP 1974

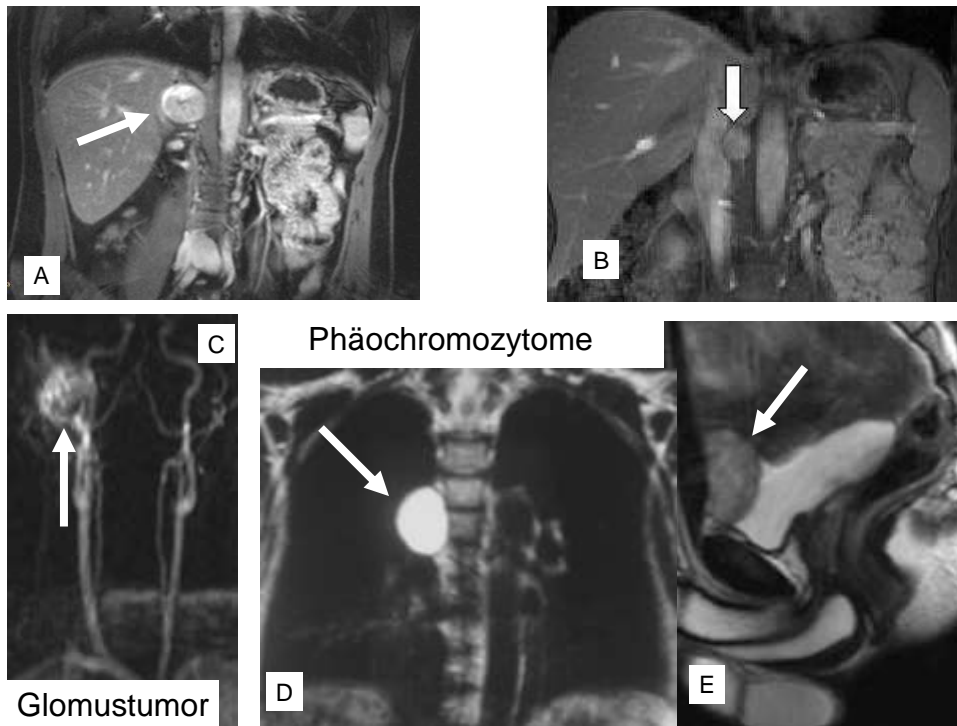


**Figure 2:** Pheochromocytoma of the left adrenal gland. Frontal view. Left: CT of the thorax, abdomen, and pelvis with contrast reagent. Right: [<sup>18</sup>F] DOPA-PET of the same body regions. The tumor of the left adrenal gland, liver, kidneys with increased contrast of the renal pelvis, and background activity are visible.



**Figure 3:** Pheochromocytoma of 7 cm in diameter of the adrenal gland. Top: resected tumor, cut in the middle and opened. Bottom: Histological section. Tumor tissue is located in the bottom 2/3 of the image, above normal tissue of the adrenal gland surrounded by lighter fat tissue (left and right).

to the elevated level of these hormones in the blood. The symptoms are multifaceted and in particular affect the cardiovascular system. Elevated blood pressure is the most common sign. Massive elevation of the blood pressure in extreme cases can be life-threatening and could lead to cardiac failure and brain hemorrhage (stroke).



**Figure 4:** Pheochromocytoma and glomus tumors in radiological images.

Top left: Pheochromocytoma of the right adrenal gland (arrow). MRI, front view. Top right: Extra-adrenal Pheochromocytoma (arrow). MRI, front view. Bottom left: Glomus tumor of the glomus jugular (arrow). Angiography. Front view. The outgoing large blood vessels originating from the aorta going to the arms and head-neck region are visible at the bottom; on the right side is the round tumor with ample blood supply. Bottom middle: Pheochromocytoma of the thorax (arrow). MRI, lateral view. The round tumor is located at the bottom end of the thorax in front of the spine. Bottom left: Pheochromocytoma of the bladder (arrow). MRI, lateral view. The bladder filled with contrast reagent is visible behind the tumor. A from Neumann HP et al *Ophthalmologie* 2007;104:119–126 with kind permission of the publisher, D from Bender BU et al *J Clin Endocrinol Metab* 1997 with kind permission of the publisher (for complete reference please see section references).

Pheochromocytomas are rare tumors. These tumors may be familial or non-familial. The majority, approximately 90%, originate in the adrenal glands. Extra-adrenal pheochromocytomas are mainly found in proximity of the adrenal glands or along the large arteries close to the adrenal glands. Pheochromocytomas located in the chest area, called thoracic pheochromocytoma, are very rare. Pheochromocytomas occur with similar frequency in both genders. The typical age of diagnosis is between 30 to 50 years.

Glomus tumors (Figure 3, 4) are tumors of the paraganglia in the area of the base of the skull and the neck. These paraganglia are named after their specific location, glomus caroticum, jugulare, tympanicum, or vagale, and these tumors are called glomus caroticum tumor etc.

### *Nomenclature*

The nomenclature for pheochromocytoma and glomus tumors is not well defined. In this brochure we use the common nomenclature used by most physicians. The nomenclature released by the World Health Organization (WHO) differs slightly from the nomenclature used in this brochure.

Pheochromocytoma is named after the staining pattern with chromium salt (phéo= appearance, chromo= stains with chromium, cytoma= abnormal cell growth, tumor). The WHO pheochromocytoma limits the use of term pheochromocytoma to tumors of the adrenal glands. In this brochure we did not use the definition by the WHO, but rather the broader definition of pheochromocytoma. Clinicians define pheochromocytoma not just by the location and histology (features seen under the microscope) of the tumor, but also with the accompanying signs and symptoms such as high blood pressure, fast pulse, excessive sweating, and headache. These symptoms also apply to extra-adrenal pheochromocytoma. These tumors are often called extra-adrenal pheochromocytoma of the abdomen, thorax, or pheochromocytoma of the urinary bladder.

Paraganglioma. Paraganglioma refers to tumors of the paraganglia and could be used for all tumors of the paraganglial system. The WHO limits the term paraganglioma to all extra-adrenal tumors.

According to this classification glomus tumors are also paraganglioma. The WHO nomenclature explains designation such as thoracic paraganglioma, head and neck paraganglioma. The term paraganglioma will not be used in this brochure.

The paraganglial system consists of the sympathetic and parasympathetic nervous systems with opposing functions. Sometimes the old staining characteristics of the tissues are used to classify the tumors: sympathetic = chromaffin tumors; parasympathetic = non-chromaffin tumors. The tumors of the sympathetic nervous system usually become symptomatic due to release of high levels of adrenaline and noradrenaline. These tumors are also called secreting (or functional) paraganglioma. Tumors of the parasympathetic nervous system (i.e. tumors of the base of the skull, neck, and the chest) are usually non-secreting tumors, hence their designation as non-secreting paraganglioma.

#### **4. HOW DANGEROUS IS A PHEOCHROMOCYTOMA?**

Before we describe the risks of a pheochromocytoma in detail we would like to describe the course of the disease. Many post-operative pheochromocytoma patients had a long history of the disease. Usually the patients presented to their primary care physicians with abdominal pain starting at a relative young age. The symptoms were not specific, i.e. they didn't lead directly to the diagnosis of a tumor of the adrenal glands with secretion of stress hormones. Rather malaise, chest pain, and/or excessive sweating were described. Echocardiograms were usually performed but did not show any abnormalities. Many patients had a normal or elevated blood pressure that did not draw special attention and was treated with medication for high blood pressure (such as beta-adrenergic blockers). If symptoms persisted patients were usually referred to a cardiologist, and a baseline electrocardiogram (ECG) or an exercise ECG were performed. Some patients underwent coronary angiography. In most cases no abnormalities of the heart were detected. Some patients were referred to psychiatrists in particular when the patients also suffered from anxiety. Extraordinary events may have been such as the patient's insistence on further examinations, a second opinion, or a substitution of the primary care physician that led to a reevaluation and revision of the original diagnosis. Initiated by the primary care physician or referred specialists, some patients underwent ultrasound examinations of the abdomen or computed tomography or MRI that led to the detection of the tumor. Lastly, a combination of blood and urine tests for catecholamines metanephrines and the detection of the tumor by ultrasound, CT, or MRI led to the diagnosis. With the diagnosis or the strong suspicion, suddenly the assessment and recommendation of the physicians changed. Now the patients were

informed of a dangerous tumor, were admitted to the hospital, and were prepared for operation seemingly under time pressure. Suddenly they became a really interesting case and surgeon and anesthesiologist came immediately and prepared the patient for the surgery. Tumors were mostly resected with a “sufficiently large” abdominal cut, usually explained by the necessary overview of the area to resect such a dangerous tumor. Post operation most patients were informed that the histological examination of the tumor showed that the tumor was benign. More recently, patients were given a score according to the Thompson Score (see chapter 10) which sometimes adds to the confusion about benignity and prognosis. The recommendations for follow-up care, if given at all, were usually limited to the measurements of catecholamines whereas genetics was rarely mentioned.

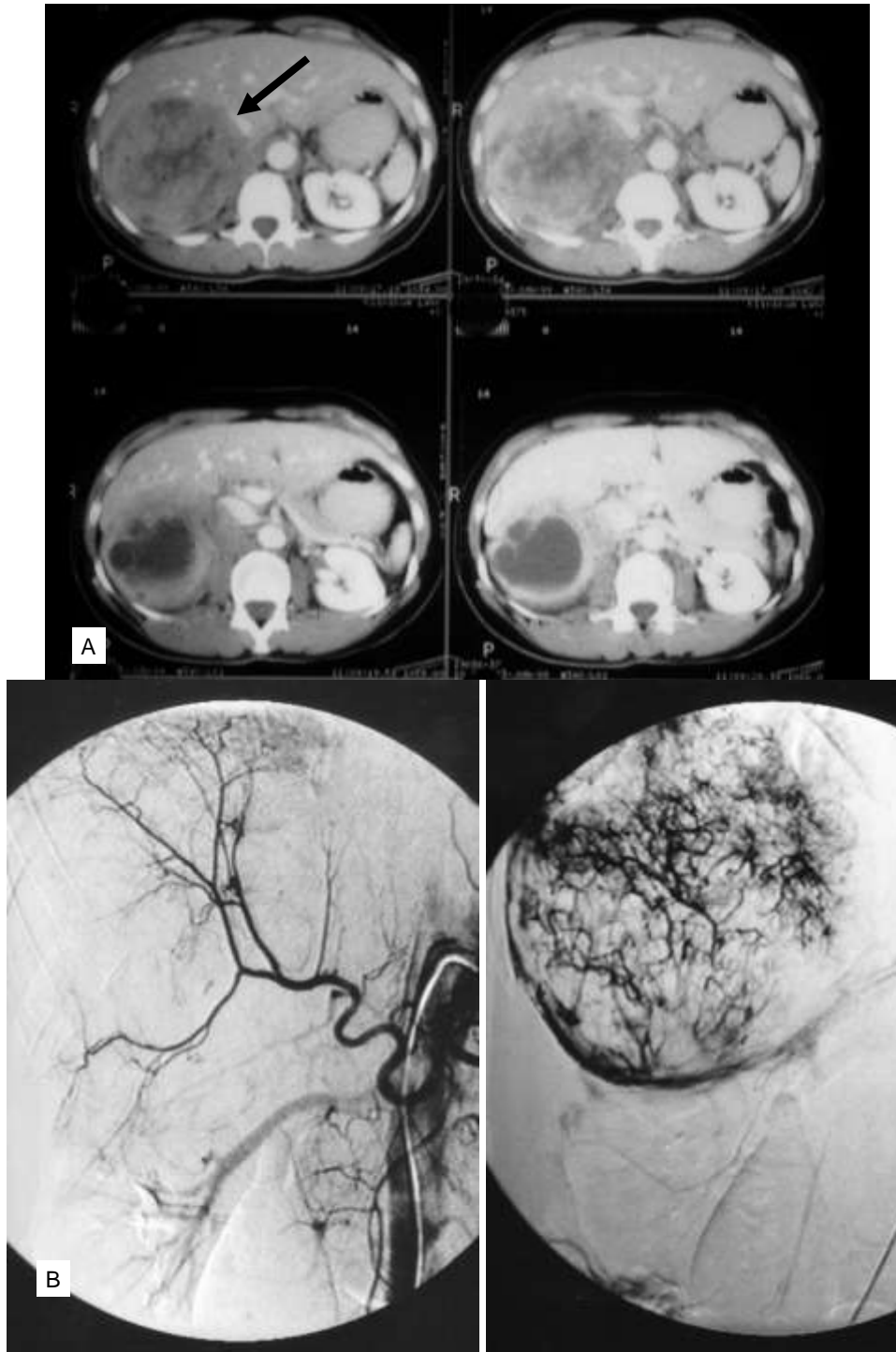
This summarizes the typical course of pheochromocytoma and describes the danger of pheochromocytoma.

1. Pheochromocytoma produces the stress hormones adrenaline and noradrenaline and secretes these in uncontrolled, non-predictable intervals and in different amounts into the blood stream. This leads to the symptoms of rapid heart rate, headache, and excessive sweating as well as episodic or permanent high blood pressure. The surgery removes the tumor and the related symptoms and high blood pressure. These tumors that mostly affect young and otherwise healthy adults (experience of the Freiburg’s International Pheochromocytoma Registry) may become life-threatening usually in a sudden manner. However, life-threatening complications are nowadays rare cases. They are usually preceded by symptoms over a long period and changes in blood pressure. Frequent

palpitations and excessive sweating, hot flashes within a few days usually precede a possible cardiac failure or stroke. Special constellations could lead to a sudden crisis, for instance when a tumor is not recognized as a pheochromocytoma before the surgery and the palpation of the tumor by the surgeon leads to massive release of the hormones.

2. The question, if intravenous injection of contrast agent is dangerous, is raised frequently. Years of experience in the Department of Radiology of Freiburg University have demonstrated that this is not the case. There are no scientific reports of such but even coronary angiographies (recorded in our registry) did not cause complications. However, tumor angiography may be dangerous, i.e. the examination that is used to define the abdominal organ as the origin of the tumor (Figure 5).
3. Prior to the surgery the blood pressure should be stabilized (see chapter 7). Alpha blocker should be used. Beta blocker should only be used in Pheochromocytoma when the heart frequency is elevated and alpha blocker has been already administered.
4. During pregnancy the risk of a sudden crisis is significantly increased due to enlargement of the placenta and movements of the fetus (see chapter 18).
5. Pheochromocytoma can be malignant in approx. 5% of cases and malignant pheochromocytoma will be discussed in chapter 10 and 12.





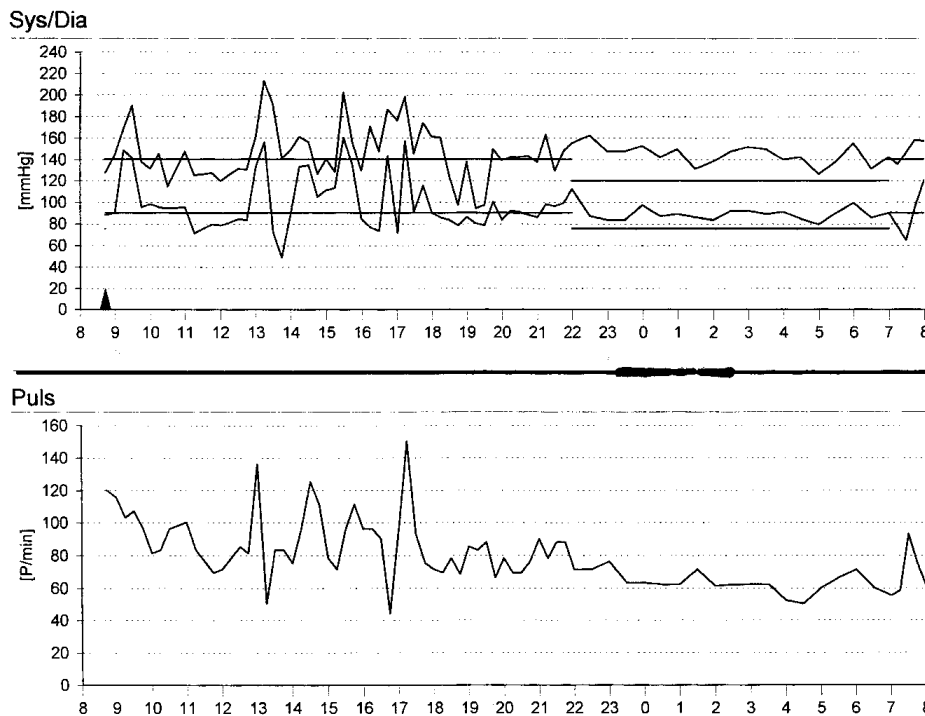
**Figure 5:** Asymptomatic tumor in the right upper abdomen (A: CT with contrast reagent) discovered during a preoperative general clarification of uterus myomatosis. No high blood pressure. During angiography (B: left: liver and adrenal glands, right: tumor. The tumor is located at the bifurcation of the location seen in the left image, left middle-top), a shock with massive high blood pressure occurred. Adrenaline in the urine was elevated to 4648 mg/day (normal: < 20) and noradrenaline to 22893 mg/day (normal: < 80). The tumor could be removed. No permanent damage occurred.

6. In summary, under normal circumstances pheochromocytoma is not life-threatening. Surgery is recommended to be done soon. Admission to the hospital is advised when acute symptoms presented within a few days.

Special circumstances in asymptomatic pheochromocytoma patients arise when the patients are carrier for mutations in the genes RET, VHL SDHD, SDHB, SDHC, SDHA, TMEM127, MAX and NF1. With the exception of patients with SDHB mutations (with not infrequent malignant pheochromocytoma) it is advised to wait until symptoms present. This should be extensively discussed with the patient. Long-term follow-up of patients support this course of action.

## 5. SIGNS AND SYMPTOMS OF PHEOCHROMOCYTOMA AND PARAGANGLIOMA

Pheochromocytoma is characterized by the effects that the produced hormones have on the body, particularly on the circulation. The heart is excited by these hormones and beats faster and stronger. Usually this happens in phases, often in bouts. The pulse could be very fast, i.e. over 200 beats per minute. Many patients can feel their heart. They are mostly seen by their primary care physicians or cardiologists. Often the ailments are not apparent during the doctor's visit and the physician can't find a cause of the patient's complaints. The blood pressure is either constantly or episodically elevated (Figure 6). Typical for pheochromocytomas are



**Figure 6:** 24-h blood pressure (systolic and diastolic, normal range are the horizontal lines) and heart frequency recording. In the top recording, short-term intense increases of the blood pressure are recognizable. In the bottom recording, multiple short increases of the pulse frequency are visible.

attacks of high blood pressure (so called intermittent hypertonia). Other signs are headaches and excessive sweating. Some patients experience hot flashes without any causes and have to change their clothing. These hot flashes are very irregular sometimes only once in several weeks, but sometimes they occur daily or several times per day. The list of symptoms for pheochromocytoma is long. The attacks could lead to anxiety and panic. Often they are accompanied with paleness of the face and enlarged pupils. Fatigue, weight loss, urge to urinate, and diarrhea as well as elevated blood sugar (diabetes mellitus), abnormal heart rhythm or heart failure can occur (Table 1). Symptoms in non-inherited pheochromocytoma patients are undistinguishable from symptoms in patients with inherited pheochromocytoma (i.e. patients with mutations in the genes RET, VHL, NF1, SDHB, SDHC, SDHD, SDHA,

Table 1: Frequent symptoms for pheochromocytomas

Headache	92 %
Hot flashes/excessive sweating	65 %
Rapid heart rate	73 %
Panic attacks	60 %
Agitation	51 %
Pain in chest, abdomen, pelvis	48 %
Nausea, vomiting	43 %
Fatigue	38 %
Weight loss	14 %

TMEM127 and MAX). All pheochromocytomas cause the described symptoms. The symptoms do not indicate a particular location of the tumor. Asymptomatic pheochromocytomas are observed more frequently during preventive check ups, for instance due to a family history of the described mutations. Such asymptomatic patients usually present with a normal blood pressure but might have elevated levels of catecholamines in the blood or urine.

Glomus tumors cause discomfort due to their location and the growth of the tumor. The glomus caroticum tumors can be palpated or are even visible (Figure 7) from the outside. Sometimes they grow inside and cause problems with swallowing. Tumors of the glomus tympanicum can lead to ear noises synchronous to the pulse or impaired hearing. Due to the confined space in the ear even relative small tumors can lead to symptoms. Glomus tumors usually don't lead to elevated catecholamines in blood or urine.



**Figure 7:** Glomus tumor of the left glomuscaroticum.

## 6. LABORATORY (BIOCHEMICAL) DIAGNOSTIC

The diagnosis of pheochromocytoma is confirmed by laboratory diagnostics and imaging. Laboratory diagnostics are performed on 24-h-urine or blood plasma.

### Normal levels of catecholamines and their metabolites

The measurements are given in grams (g), ( $\mu\text{g}$ , ng, pg) or mol ( $\mu\text{mol}$ , nmol, pmol).

Normal range for **24-h-urine** for adults (for Freiburg, and in parentheses for Dresden)

Noradrenaline: < 504 (< 473) nmol/24 h

Adrenaline: < 121 (< 109) nmol/24 h

Dopamine: < 3.2  $\mu\text{mol}$ /24 h

Metanephrine: 122-1540 nmol/24 h

Normetanephrine: 874-2846 nmol/24 h

Normal range for **24-h-urine** for adults (for Freiburg, and in parentheses for Dresden) in milli- and micro-gram

Noradrenaline: < 85.5 (< 80)  $\mu\text{g}$ /24 h

Adrenaline: < 22 (< 20)  $\mu\text{g}$ /24 h

Metanephrine: < 302  $\mu\text{g}$ /24 h

Normetanephrine: < 527  $\mu\text{g}$ /24 h

3-methoxytyramine: < 434  $\mu\text{g}$ /24 h

Normal range for measurements in **plasma** in Freiburg and Dresden:

Noradrenaline: < 460 ng/l

Adrenaline: < 90 ng/l

Metanephrine: < 70 ng/l

Normetanephrine: < 120 ng/l

The following conversions should be applied:

Noradrenaline: ng/l x 0.0059 = nmol/l

Adrenaline: ng/l x 0.0055 = nmol/l

Dopamine: ng/l x 0.0065 = nmol/l

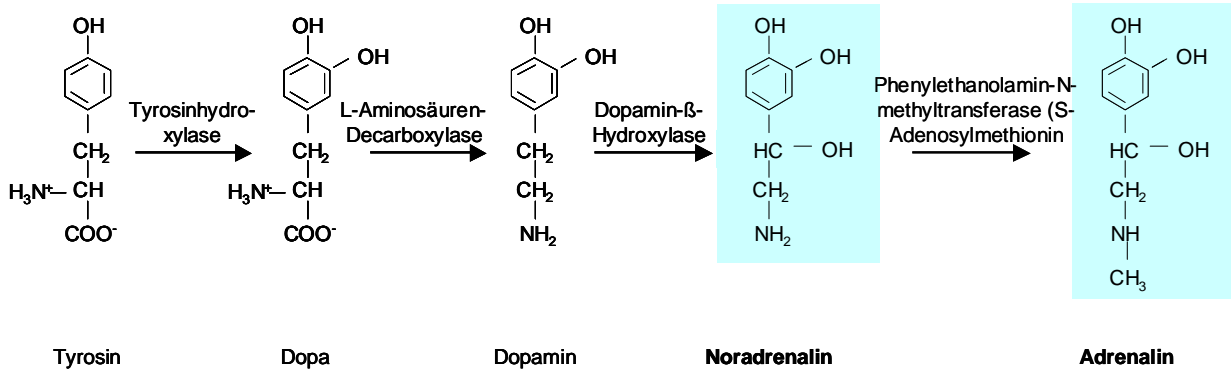
Metanephrine: ng/l x 0.0051 = nmol/l

Normetanephrine: ng/l x 0.0054 = nmol/l

### **Biosynthesis and metabolism of catecholamines**

Hormones are substances that are produced by glands and released into the bloodstream. Catecholamines are hormones that are mainly produced in the adrenal glands but also by cells of the paraganglial (sympathetic nervous) system. The catecholamines are Adrenaline and Noradrenaline. They are released by stress. They are called catecholamines because they are chemically derived from catechol (1,2-dihydroxybenzene). The medulla of the adrenal glands mainly produces adrenaline. Noradrenaline is produced mainly in nerve cells of the paraganglial system and to a lesser extent in the adrenal medulla. The biosynthesis and degradation of the catecholamines is complex and summarized in figure 8. The first step in the biosynthesis is the amino acid Tyrosine. Tyrosine is first converted into Dopa by the enzyme tyrosinehydroxylase and Dopa is then converted into Dopamine which is then converted into Noradrenaline. Up to this step the biosynthesis of catecholamines in the medulla of the adrenal glands and in nerve cells is identical. In the medulla of the

adrenal glands, Noradrenaline is then converted into Adrenaline by the enzyme phenylethanolamine N-methyltransferase.

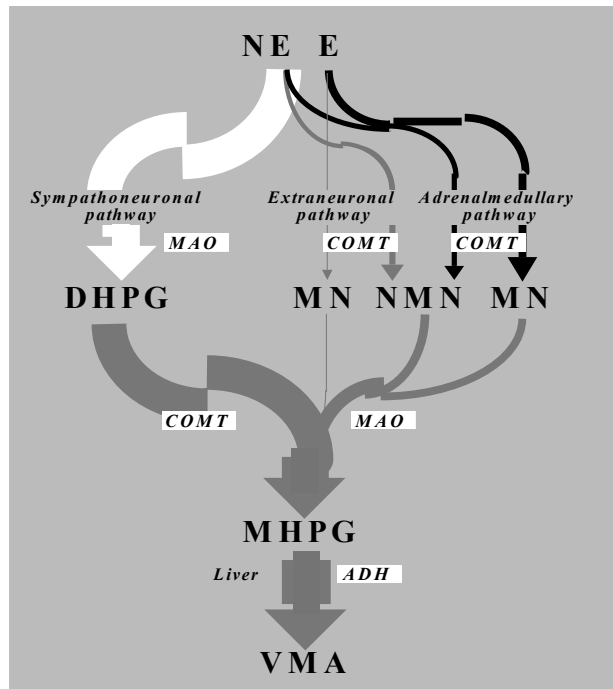


**Figure 8:** Synthesis of catecholamines.

The steps of the degradation and their enzymes are shown in figure 9. Catecholamines and their metabolites are measured in the blood by different methods (HPLC, LC-MS/MS, ELISA, RIA). Elisa and RIA are inferior to HPLC and LC-MS/MS for a reliable measurement of metanephrines. The normal range for each method may vary to some extent and therefore it is recommended to interpret the measured plasma concentrations of catecholamines and metanephrines based on the reference values for the selected method. Elevation of catecholamines and/or metanephrine may have diverse causes. Apart from certain food, medications, and endogenous factors such as



stress are of influence on the levels of plasma catecholamines and metanephrines.



not

**Figure 9:** Degradation and secretion of catecholamines. NE: norepinephrine, E: epinephrine, DHPG: 3,4-dihydroxyphenylglycol, MN: Metanephrine, NMN: Normetanephrine, MHPG: 3-methoxy-4-hydroxyphenylglycol, VMA: vanillylmandelic acid, MAO: Monoaminoxidase, COMT: Catecholamine-O-Methyl-Transferase, ADH: alcohol dehydrogenase, Sympathoneuronal: sympathetic nerves, Extraneuronal: Endothelial cells of blood vessels, cardiomyocytes, Adrenomedullary: Metabolic processes in the adrenal gland.

Slightly elevated plasma levels just over the highest value of the normal range ( known as 'grey area') do necessarily mean that there is a pheochromocytoma since several of the above-mentioned factors might be responsible for these slight elevations. The gray area, however, is not exactly defined. For noradrenaline there is such a grey area up to twofold the highest value of the normal range. In these cases the physician should discuss with the patient if medications or food might be an explanation for elevated measurements. It is recommended to eliminate/avoid them if possible and eventually to perform a Clonidine suppression test.

Factors that may lead to an elevation of catecholamines and metanephrine should be avoided to guarantee accurate determination of catecholamines and metanephrine in 24-h-urine or plasma. Medications such as tricyclic antidepressants, MAO inhibitors, methyl-dopa, and stimulants should be weaned off and food such as tea, bananas, and almonds should be avoided.

Urine should be collected in a container containing 10% hydrochloric acid. The purpose of this acid is to prevent degradation of catecholamines and metabolites. Some laboratories do not add the acid anymore to the containers but add an acid to the urine at the time when the urine arrives in the lab. So this is done before prolonged storage of the urine sample.

In case of blood sampling, a forearm venous canula should be placed and a blood sample should be drawn after at least 20 minutes rest of the patient in the supine position. After the blood draw, the sample should be chilled immediately on ice until the sample arrives in the laboratory.

### ***Clonidine suppression test***

Clonidine is a drug used for treatment of hypertension. It inhibits the release of adrenaline and noradrenaline. Clonidine's effect on reducing circulating noradrenaline is used as an investigative test for pheochromocytoma. In a clonidine suppression test, plasma normetanephrine levels are measured before and 3 hours after a single 300 mg oral dose has been given to a patient. A negative test (a proper decrease of plasma normetanephrine) excludes the presence of a pheochromocytoma. The clonidine

suppression test can be done on an outpatient basis. However, since clonidine can cause slight drowsiness, the patient should not drive self home by car.

## **7. IMAGING**

Ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), or diagnostic nuclear medicine, i.e. MIBG scintigraphy, Octreoscan, DOTATATE-PET, DOPA-PET and FDG PET are all used to diagnose pheochromocytoma. Diagnostic nuclear medicine can be combined with CT or MRI, e.g. DOPA-PET-CT.

### ***Ultrasonography***

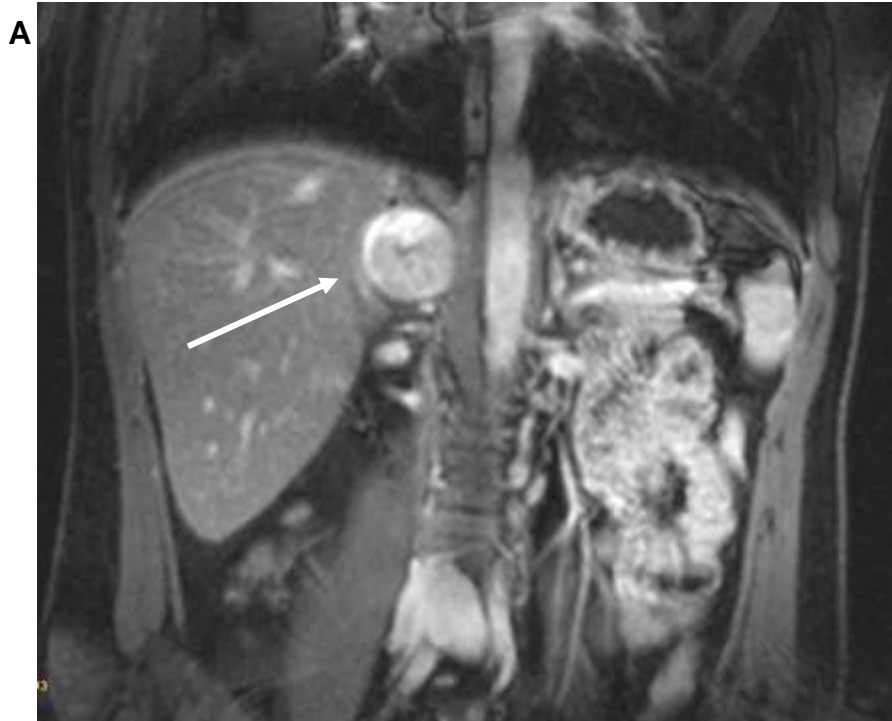
Ultrasound is very common and available. Most patients with pheochromocytoma received ultrasound of the abdomen in case of presentation with nonspecific abdominal pain. However, pheochromocytomas are usually located in the posterior of the abdomen resulting in rather low sensitivity of ultrasound. We have demonstrated a sensitivity of approx. 40% in 1993. Sensitivity can be much higher if the ultrasound is performed by experienced physicians.

### ***Computed tomography (CT)***

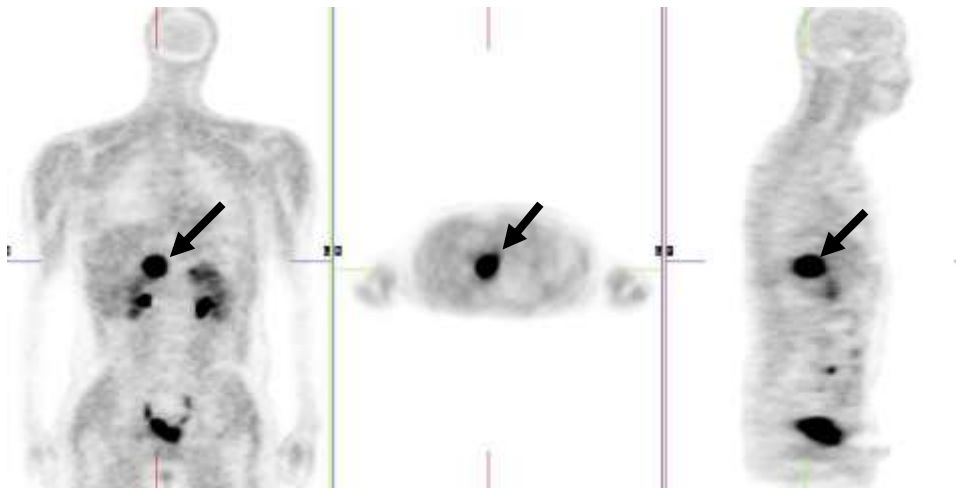
The CT scan is performed with contrast agent. The serum creatinine ought to be determined prior to CT scan. The contrast agent may worsen kidney damage in patients with preexisting kidney insufficiency. Therefore, a CT scan is not advised if the serum creatinine is above 1.5 mg/dl. Contrast agent may also induce hyperfunction of the thyroid. The TSH levels ought to be measured prior to CT scan. The CT scan produces transverse cross sections (i.e. horizontal cross sections of standing person) of the body. The resolution of the CT is 1-2 mm.

### ***Magnetic resonance imaging (MRI) (figure 4a, b, d, e, 10a, 11)***

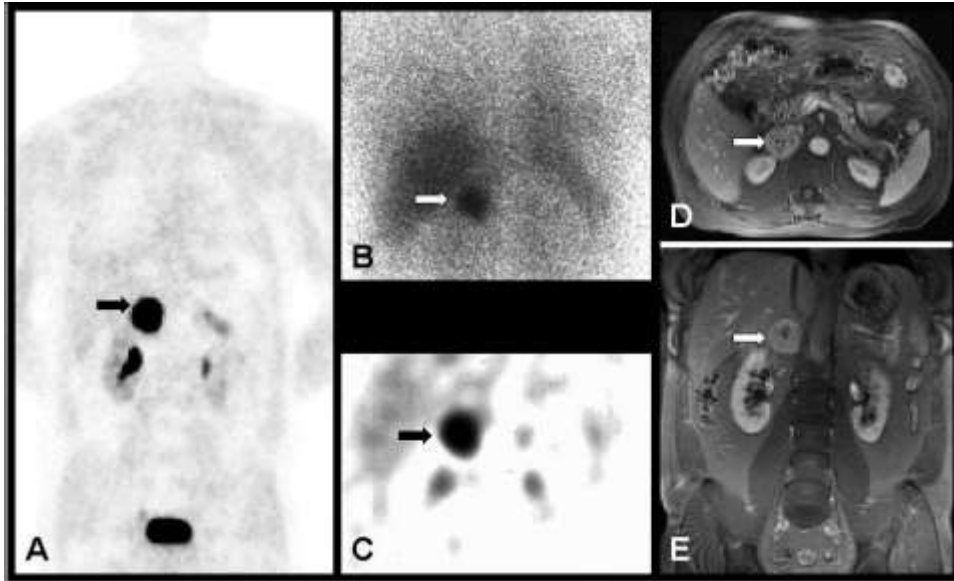
MRI is also performed with contrast reagent but the risk for kidney damage is reduced compared to the CT contrast agent. MRI is not advised if the serum creatinine is above 1.5 mg/dl. MRI scanners produce a loud noise and headsets are advisable. The patient is placed in a small confined room and it takes 20-40 min to complete a MRI. Quite a few patients, in particular children and patients suffering from claustrophobia, feel uncomfortable during an MRI and a sedative might be given. The MRI constructs images based on differences in certain properties of different tissues resulting in different contrasts ( $T_1$  and  $T_2$  relaxation times).  $T_2$  images show a remarkable hyperintensity of pheochromocytomas and paragangliomas. Contrast agents are given right before the scan which further improves the detection of structural differences. The MRI produces transverse cross sections, frontal cross sections and side cross sections of the body. Frontal cross sections allow for complete visualization of tumors (with a resolution of 5 mm, tumor is visible on 8-10 images) in the posterior abdomen (so called retroperitoneum) where more than 95% of the pheochromocytomas are located.



B



**Figure 10:** Pheochromocytoma of the left adrenal gland. MRI (A) and [ $^{18}\text{F}$ ] DOPA-PET (B). The [ $^{18}\text{F}$ ] DOPA-PET depicts the tumor (arrows) in frontaland lateral view. Kidneys and strong contrast in the renal pelvis and the bladder are visible. From Neumann HP et al *Ophthalmologe* 2007;104:119–126 with kind permission of the publisher



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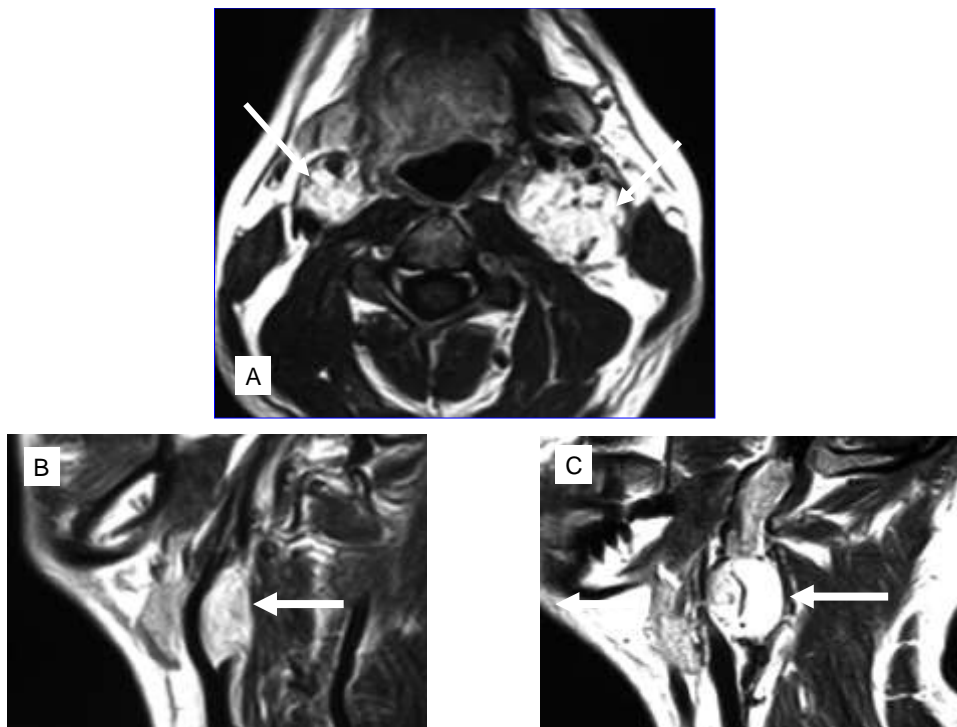
**Figure 11:** Imaging of the same pheochromocytoma by [ $^{18}\text{F}$ ] DOPA-PET (A), MIBG scintigraphy (B), SPECT (C), MRI in horizontal (D) and frontal (E) projection. The better quality of [ $^{18}\text{F}$ ] DOPA-PET compared to MIBG and SPECT can be appreciated. From Hoegerle S et al Radiology 2002; 222:507–512 with kind permission of the publisher (for complete reference please see section references).

### ***Diagnostic nuclear medicine (Figures 10, 11, 14)***

Diagnostic nuclear medicine is usually used to confirm tumors detected by MRI or CT and to exclude multiple pheochromocytomas and depicts the functional characteristics of pheochromocytoma and paraganglioma. A number of substances are available for nuclear medicine imaging of these tumors.

Mostly [ $^{123}\text{I}$ ] MIBG is used for scintigraphy (Figure 11). A positive finding is usually indicative for an adrenal or extra-adrenal pheochromocytoma. In the case of malignant pheochromocytoma [ $^{123}\text{I}$ ] MIBG scintigraphy allows for the detection of metastasis. Very small pheochromocytomas are occasionally not detected due to the resolution limit.

Patients are given oral perchlorate at least 30 min. prior to [ $^{123}\text{I}$ ] MIBG injection to prevent the uptake of radioactive Iodine-123 into the thyroid. The imaging is performed 4 and 24 hours after injection thus requiring two appointments. Another disadvantage is the interference of many medications with [ $^{123}\text{I}$ ] MIBG. These medications should be stopped if possible. Medications that interfere with [ $^{123}\text{I}$ ] MIBG include several blood pressure and heart medications as well as antidepressants.

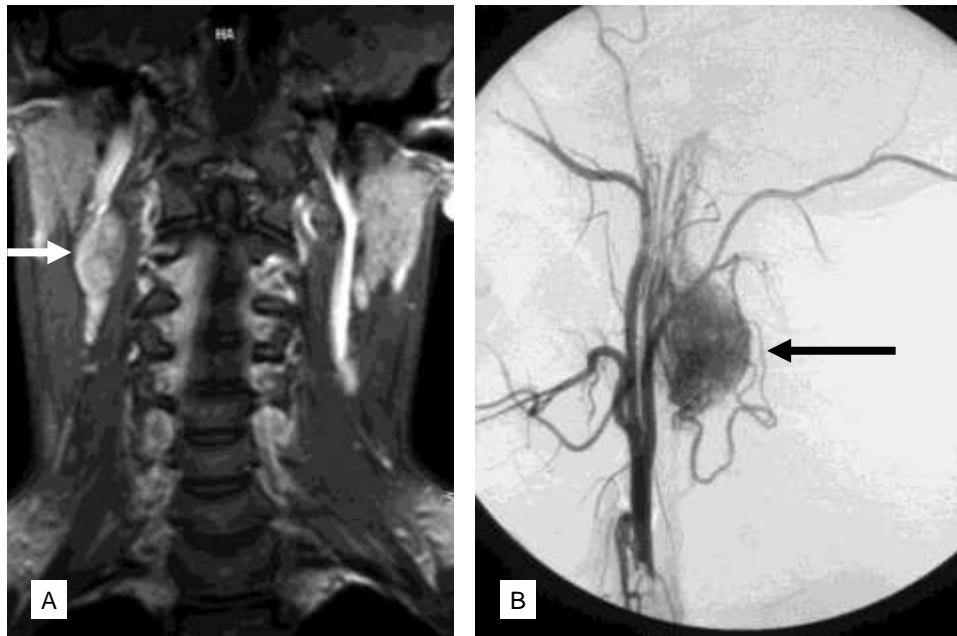


**Figure 12:** Bilateral tumor of the glomus caroticum depicted by MRI in horizontal (A) and lateral (B, C) projection.

[ $^{18}\text{F}$ ] DOPA-PET (Figures 10b, 11) and [ $^{18}\text{F}$ ] DOPA-PET/CT produce much more accurate images and are available in larger medical centers. [ $^{18}\text{F}$ ] DOPA is a precursor of the hormones produced by pheochromocytoma and is incorporated into these hormones and enriched. Pretreatment of the thyroid is not necessary and the examination only takes 90 min from injection to the completion of scan. In comparison



to [ $^{123}\text{I}$ ] MIBG scintigraphy and SPECT [ $^{18}\text{F}$ ], DOPA-PET offers a higher contrast and higher resolution allowing the detection of very small pheochromocytomas. Alternative diagnostic nuclear medicine such as Octreoscan, [ $^{68}\text{Ga}$ ] DOTATOC-PET, and [ $^{68}\text{Ga}$ ] DOTATATE-PET/CT are rarely used. One exception is the diagnosis of the malignant pheochromocytoma (see chapter 12). For metastatic pheochromocytoma, [ $^{18}\text{F}$ ] FDG PET is very sensitive for tumor detection, especially in case of an underlying SDHB mutation.



**Figure 13:** Tumor of the glomus vagale. Depiction by MRI (left) and angiography (right).

The combination of MRI and diagnostic nuclear medicine is particularly important for pheochromocytoma in the thorax or the pelvis. Examples for pheochromocytoma in the

posterior thorax (figures 18, 30), in proximity of the heart (figures 19, 57, 58), or the pelvis (figures 3e, 17) are given.

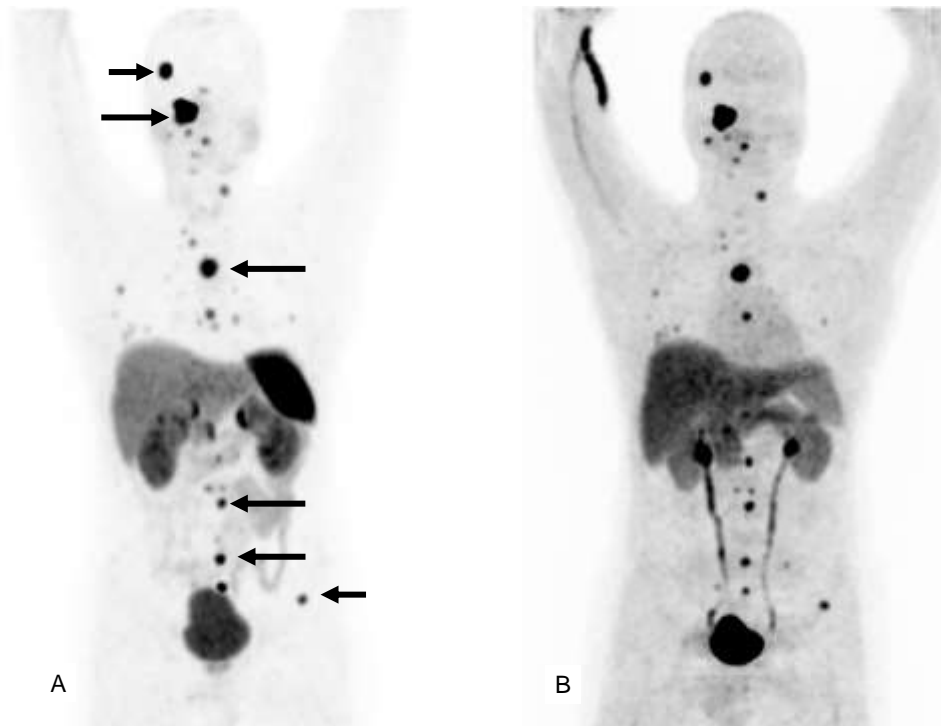
### ***Imaging for glomus tumors***

The same imaging methods could be used for imaging of glomus tumors. Additionally, [<sup>68</sup>Ga] DOTATOC-PET/CT, and [<sup>68</sup>Ga] DOTATATE-PET/CT give good results for the exclusion of multiple glomus tumors.

*Ultrasound* allows the detection of enlarged structures in the neck area. However, the differentiation between enlarged lymph nodes and glomus tumors is occasionally difficult.

MRI is currently the gold standard for imaging of glomus tumors. Contrast agent is given intravenously. Tumors of the glomus caroticum are depicted in Figures 12 and 20, tumors of the glomus jugulare and tympanicum are depicted in Figure 21, and tumors of the glomus vagale are depicted in figure 13.

[<sup>18</sup>F] DOPA, [<sup>68</sup>Ga] DOTATOC-PET/CT, and [<sup>68</sup>Ga] DOTATATE-PET/CT are comparable tests for the depiction of glomus tumors. The duration for these imaging methods is between 90 min and 2 hours. The patient's body is scanned from the head to the pelvis. These imaging methods (i.e. PET/CT) are superior for imaging of multiple tumors or metastasis compared to other methods (Figure 14).

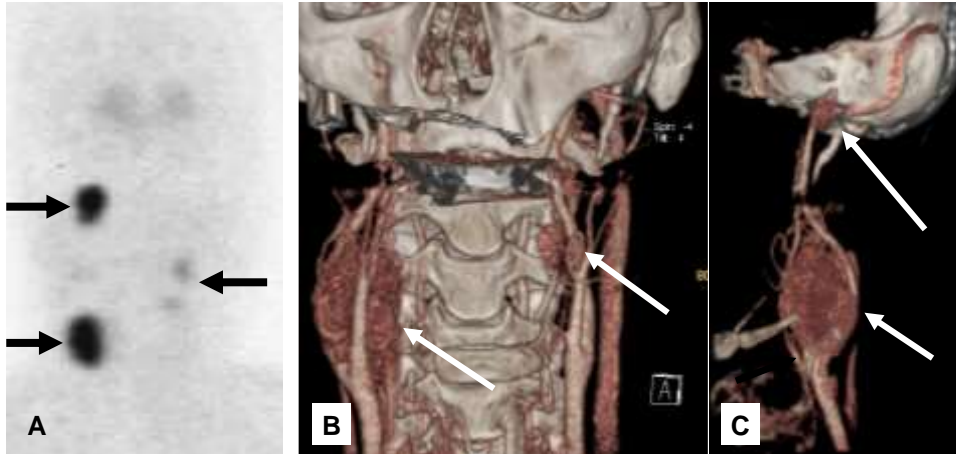


**Figure 14:** Malignant glomus tumor. A: [ $^{68}\text{Ga}$ ] DOTATATE-PET, B: [ $^{18}\text{F}$ ] DOPA-PET. The tumor metastases (black round points in the head, thorax, and between the ureter) are similarly detectable with both methods. Some of the metastases are marked by arrows in the left image and are consistent with the ones in the right image.

MRI angiography- or CT angiography (Figure 15) are additional methods for the diagnostic of these tumors.

### Organizational considerations

The multitude of hormone-based and imaging methods for diagnosis raised the question which method(s) should be used for diagnostic purposes. Hormone-based tests and imaging are complementary for the diagnostic of pheochromocytoma. The methods of nuclear medicine are used to confirm the diagnosis and to exclude multiple tumors as a preparation for surgery.



**Figure 15:** Imaging of three tumors in the base of the skull and head by angiography-CT. [ $^{18}\text{F}$ ] DOPA-PET (A) and angiography-CT (B, C) with tumors of the glomus jugulare (A and C) and bilateral glomus caroticum (A, B, and C). From Hoegerle S et al *Eur J Nucl Med Mol Imaging* 2003;30:689-94 with kind permission of the publisher (for complete reference please see section references).

Organizational considerations include the manufacturing and delivery of the radionuclides for planning of the examination as well as the duration of the tests. The MIBG-scintigraphy takes 24 hours while the DOPA PET only takes 1 hour. Furthermore, measurements of catecholamines are not available daily.

## **8. PREOPERATIVE MANAGEMENT OF PHEOCHROMOCYTOMAS AND GLOMUS TUMORS**

Pre-operative preparations include complete blood count, blood coagulation tests, and ECG. Additionally, blood pressure should be normalized. Long-term blood pressure measurements should be performed. Alpha blockers have an important role in the treatment of blood pressure because they block the actions of catecholamines. Alpha blockers dilate the blood vessel and therefore are at risk to induce a collapse when the blood pressure becomes too low. Patients should be closely monitored especially in the beginning of the treatment and patients should drink plenty of fluids. It is recommended to encourage the patient to drink 1 liter of fluids during the first 30 to 60 minutes, later 3 liters per day. Alpha blocker should be given at an initial dosage of 10 mg phenoxybenzamine (trade name: Dibenzylin) 3 times a day. An increase to 20 mg or 30 mg 3 times a day usually leads to normalization of the blood pressure.

The manipulation of these catecholamine-producing tumors may lead to excessive release of these hormones during the surgery. Phenoxybenzamine is traditionally used to prevent severe increases of blood pressure during the surgery. It is recommended to start the medication one week before surgery. In the case of sustained rapid pulse it is recommended to add a beta blocker but only after the alpha blocker treatment has been started. A 24-hour blood pressure profile should demonstrate a normal blood pressure prior to surgery.

However, the effect of medicinal preparation for the surgery has not been conclusively proven. Even with medication (even with high dosage) there is a possibility of severe

blood pressure increase during the surgery making the medicinal preparation questionable. Unfortunately, there are no clear answers as of now if patients should be treated or not and some physicians recommend treatment while others object given the patient is normotensive.

The surgery is performed under general anesthesia. Often, prior to the surgery, a catheter is inserted into the wrist artery to allow constant monitoring of the blood pressure during the entire surgery. A second catheter is inserted into the central vein at the neck to regulate the blood pressure if catecholamines are released. These catheters are being used by the anesthesiologist at the first signs of blood pressure increases to inject fast and effective medications to prevent a blood pressure crisis.

In experienced medical centers the post-operative observation in the ICU takes only 2-3 hours after which the patients may be transferred to the inpatient units. Only in rare cases the patients are observed for 24 hours in the intensive care unit.

## **9. SURGERY OF PHEOCHROMOCYTOMA**

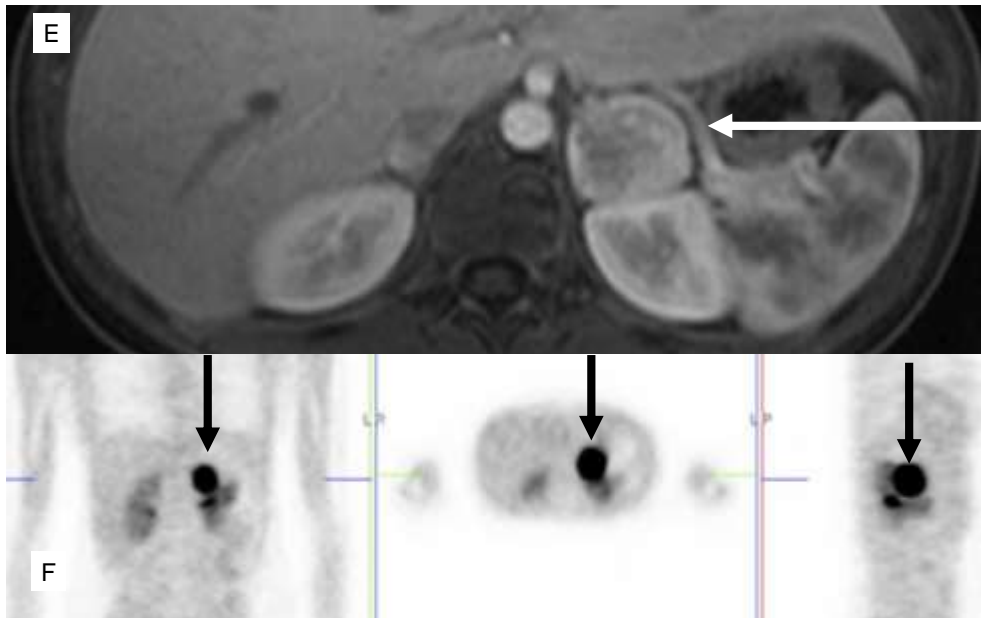
### *Tumors of the adrenal glands*

The surgery of pheochromocytoma has changed dramatically over the last few years. The introduction of minimally invasive surgery was a milestone in the surgical treatment of pheochromocytoma (Figure 16). Most of the pheochromocytomas are located in one of the adrenal glands or in close proximity of the adrenal glands (extra-adrenal retroperitoneal) and the entry point is either through the abdomen (i.e. laparoscopic) or from the back (i.e. retroperitoneoscopic). Endoscopic surgery requires sufficient practice and should only be performed by experienced surgeons specialized in minimally invasive surgery of the adrenal glands. It is important to mention that minimally invasive surgery should be utilized for almost all pheochromocytomas (independent of size and location), making open surgery obsolete today. Even extra-adrenal pheochromocytomas have been successfully removed by endoscopic techniques. Though minimally invasive surgery of the adrenal glands has to be performed under general anesthesia, fast recovery from surgery is most likely as postoperative pain is only very limited. Thereby, oral intake and full mobilization is possible from day of surgery. The average hospital stay is only 3-5 days. Complications as infection or bleeding are very rare. Scientific reports demonstrated that operating time of minimally invasive adrenalectomy is not prolonged compared to traditional open operation. Endoscopic adrenal surgery is performed with 3-5 small skin incisions of 5-10 mm length for the camera and the surgical instruments. In case of difficulties in finding the tumor, an endoscopic ultrasound may be inserted as well. Further advancement of endoscopic surgeries has led to the one-incision endoscopic removal of tumors (so-

called: SARA-technique); by this approach all instruments are inserted through one minimal incision (Figure 16).







**Figure 16:** Scars after open (A, D) and endoscopic (B, C) surgeries of pheochromocytoma. A: Scar after two surgeries of pheochromocytoma of the adrenal glands. B: Scars after bilateral endoscopic surgery from the back. C: Condition after endoscopic removal of a pheochromocytoma located below the left adrenal gland (same patient as in E). D: Condition 10 years after open surgery of a bilateral pheochromocytoma of the adrenal glands in the father. E: MRI, view from the top and [ $^{18}\text{F}$ ] DOPA-PET (F), frontal, top, and lateral view. Same patient as in C.

Whenever possible surgical removal of pheochromocytoma of the adrenal glands should be performed with preservation of the adreno-cortical function.(so-called adrenal sparing surgery or partial adrenalectomy). This means that the tumor should be removed without the normal adrenal gland tissue. This type of function-preservation surgery is of outstanding importance in patients with bilateral pheochromocytomas. In such a case as much normal tissue as possible should be preserved on both sides. As minimally invasive surgery offers a magnification of organs and tissue, endoscopic adrenal surgery facilitates a distinction between normal adrenal tissue and adrenal tumor. Thereby, adrenal-function preserving surgery is relatively easy to perform endoscopically in experienced hands. Meanwhile it has been shown that in 9 out of 10 patients with bilateral pheochromocytomas a sufficient cortisol production can be achieved by partial adrenalectomy. Only in the case of very big bilateral tumors (e.g. > 6-8 cm) function preserving surgery may be impossible.

After bilateral surgery, cortisol release should be tested by the ACTH test (see follow-up care, Chapter 11) to confirm that the adrenal glands produce sufficient amounts of cortisol.

### **Follow-up/Secondary surgery**

Secondary surgeries for local recurrent pheochromocytomas are a particular challenge to the surgeon. The scars on the operating field which develop after the first operation make the second procedure more difficult. It has been proven that the best access in the

second operation is the endoscopic approach from the back. This so-called retroperitoneoscopic technique allows a safe removal of a recurrent pheochromocytoma.

### **Tumors during childhood**

Pheochromocytoma and glomus tumors are very rare in children. However, they are located at the same areas as in adults and adolescents. Surgery is very challenging because of the patients' sizes but should always be performed in minimally invasive techniques.

### **Extra-adrenal abdominal tumors and tumors of the bladder**

The majority of the extra-adrenal tumors are located in close proximity of the adrenal glands or the big abdominal vessels, i.e. the aorta and/or the main vein (inferior vena cava) (Figures 4B, 17, 51). Some tumors are also located between these blood vessels. These operations are always a challenge for the surgeon. He/She has to decide which type of approach is the best and safest (open or endoscopic surgery). Factors influencing these decisions are the tumor size, number of tumors (single vs. multiple tumors), or biological behavior of the tumor. Without doubt, a minimally invasive operation offers the same advantages for patients with extra-adrenal pheochromocytomas as for those with adrenal pheochromocytomas as minimal

postoperative pain and fast recovery. But as these operations are really rare and difficult they should only be performed in highly experienced centers.

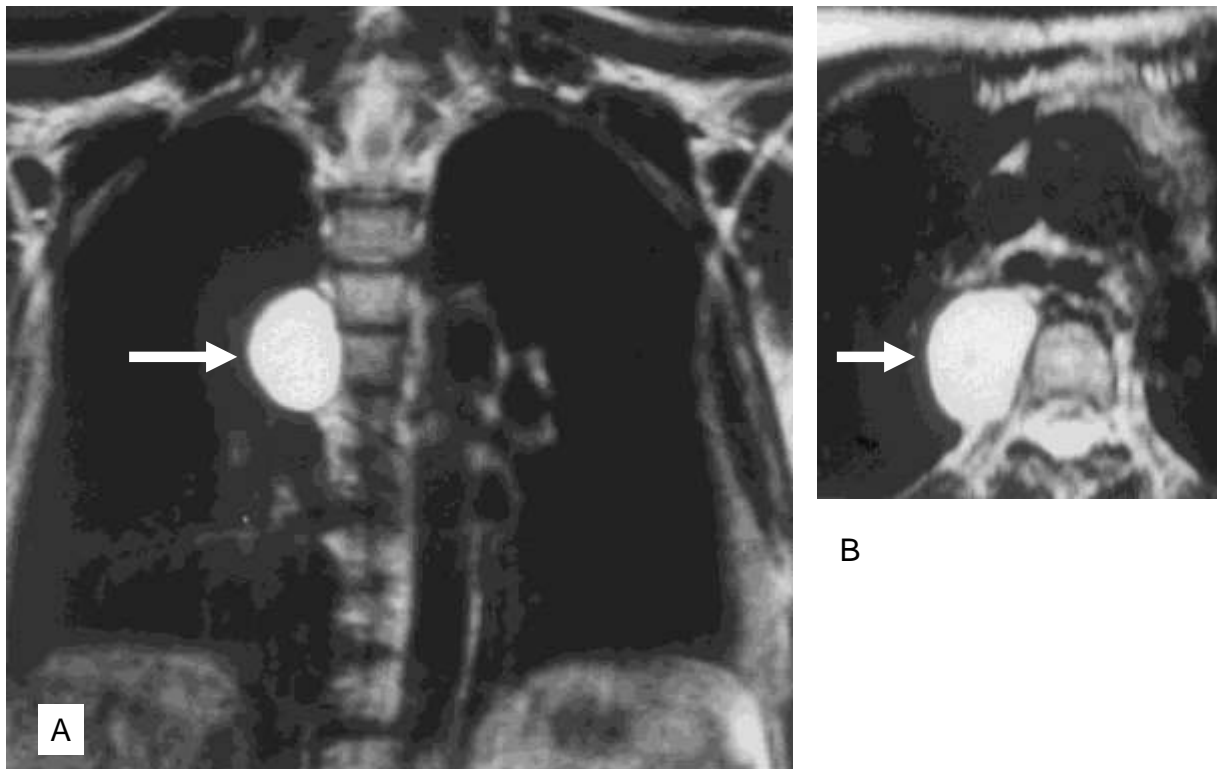


**Figure 17:** Pheochromocytoma of the bladder. CT in horizontal projection: top in the image corresponds to the front and bottom of the image to the back. The tumor (arrows) expands from the back into the bladder.

Pheochromocytomas of the urinary bladder (Figures 4E, 17) are very rare. Traditionally, these tumors are removed during open surgery. A hole is being cut into the bladder and the edges are sutured together. In some cases endoscopic surgery may be possible but this has to be restricted to selected patients.

## Tumors of the chest cavity

Pheochromocytoma of the thorax are either located in the posterior chest cavity in the area of the so-called sympathetic trunk, or in close proximity of the heart, the so-called mediastinum. Examples for tumors of the sympathetic trunk are depicted in Figures 18, 61, and an example for a tumor in proximity of the heart is depicted in Figure 19.

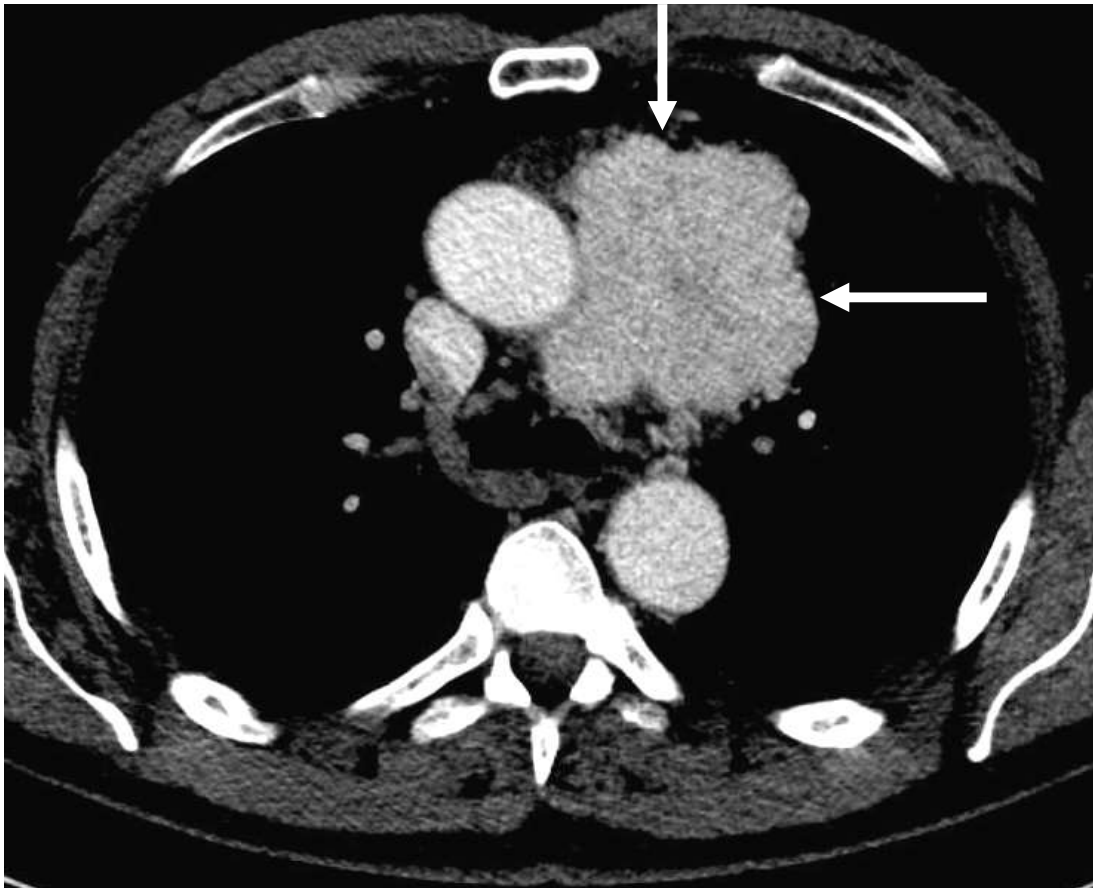


**Figure 18:** Pheochromocytoma of the thorax (arrows). Front (left) and horizontal (right) view. The tumor is located in the back region of the thorax, at the right side of the spine in the region of the sympathetic trunk. From Bender BU et al J Clin Endocrinol Metab 1997 with kind permission of the publisher (for complete reference please see section references).

Tumors of the sympathetic trunk may be removed during endoscopic surgery. During general anesthesia one lung is ventilated which is sufficient to guarantee oxygen

supply. The other lung collapses and provides room for the removal of the tumor. Endoscopic instruments will be inserted into this room and the tumors will be removed. When larger tumors of the sympathetic trunk are removed, it is imperative to prevent injuries to the blood supply of the spinal cord.

Tumors of the mediastinum should be removed by a heart surgeon or a thoracic surgeon. Small tumors are usually removed without complications. In large tumors (Figure 19), however, it must be considered, if the operation has a high risk for new and permanent side effects such as injury to several nerves. Some tumors may be not resectable.



**Figure 19:** Pheochromocytoma of the thorax (arrows). Horizontal view. The tumor is located in the front region of the thorax, the so-called mediastinum, in close proximity of the large blood vessels and nerves.

## **Treatment of silent pheochromocytoma**

Silent pheochromocytomas are tumors that have been diagnosed as a pheochromocytoma using imaging methods but do not cause symptoms. Such pheochromocytomas are usually identified in patients with mutations of the genes RET, VHL, SDHB, and SDHD. Pheochromocytomas in these patients are usually identified during clinical examination of an entire family, follow-up examination of a patient with prior pheochromocytoma, or patients with related tumors (e.g. medullary carcinoma of the thyroid) that have been identified as carriers of a mutation, e.g., of the RET gene that are clinically examined. Currently, there is controversy as to whether these tumors should be removed or not. In any case blood pressure measurements on several consecutive days should be performed. It is advisable to establish a 24h-blood pressure profile. All aspects should be taken into consideration. Some considerations are listed here:

1. In young women it is recommended to remove the tumor because during pregnancy the increased pressure of the growing uterus and the baby's movements might initiate symptoms or even a catecholamine crisis. This applies to all tumors of the abdomen.
2. The specific mutation could either favor or postpone a surgery. Mutations of the RET and SDHD gene very rarely lead to malignant tumors. This would argue for postponing the surgery. Mutations of the VHL gene sometimes lead to malignant tumors. It doesn't seem sufficient to recommend surgery. Mutations of the SDHB gene cause in one third of the patients malignant tumors. Removal of the tumor is recommended in these patients.

3. The catecholamines or metanephrines might be normal or elevated. The latter leads to the conclusion that the tumor releases hormones into the blood stream. It is not clear if surgery should be recommended in patients with elevated hormone levels, however, most physicians would recommend surgery.



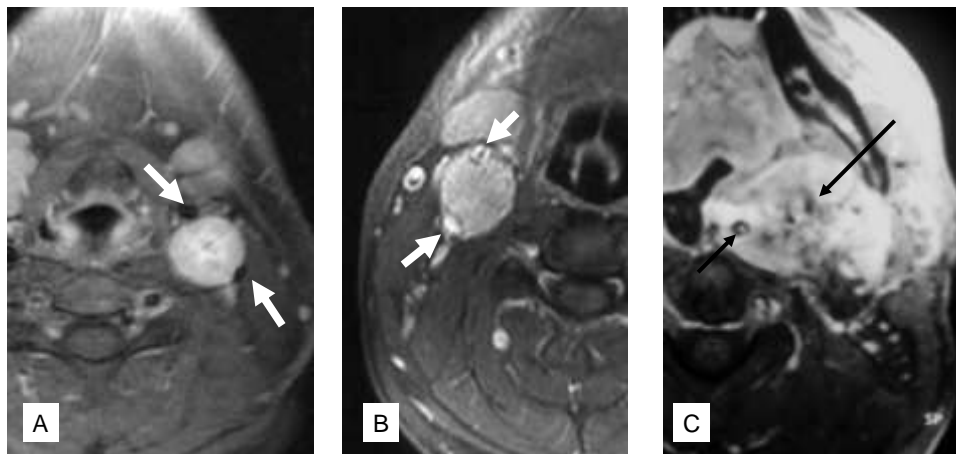
## 10. SURGERY OF GLOMUS TUMORS

Glomus tumors of the head and neck (or head and neck paragangliomas) are a well-defined group. These tumors stand out because of their spatial expansion, pressure on and infiltration of neighboring structures and absence of general symptoms such as high blood pressure or hot flashes. They are derived from the parasympathic nervous system and are only weakly stained by histological dyes (non-chromaffine). The majority of these paraganglial tumors of the nervous systems are not appreciated as such because they are usually treated by ear, nose, and throat surgeons, sometimes by vascular surgeons and neurosurgeons.

The tumors of the glomus caroticum are the most common (Figures 7, 12, 20). They are located in close proximity of the common carotid artery and the branching external and internal carotid arteries. In close proximity are also the vagal nerve and the large venous blood vessels for the head and neck. These tumors are rich in blood and have many small blood vessels like all paraganglial tumors (pheochromocytoma and glomus tumors).

There is a special classification for the expansion of the glomus carotid tumors named after the surgeon Shamblin (Figure 20): Shamblin class I (Figure 20A): the tumors are in close proximity of the large blood vessels (external and internal carotid arteries); Shamblin class II (Figure 20B): the tumors are beginning to surround the large blood vessels; Shamblin class III (Figure 20C): the blood vessels are located within the tumor and completely surrounded by the tumor.

The surgeries of the glomus caroticum tumors are extremely challenging due to their

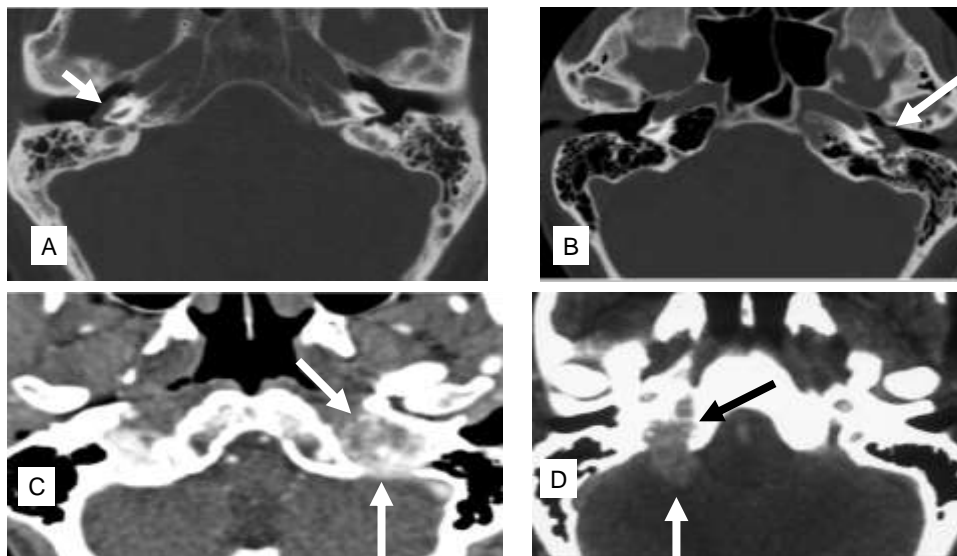


**Figure 20:** Examples of the Shamblin classification of glomus caroticum tumors. A: Left-sided tumor Shamblin class I. B: Right-sided tumor Shamblin class II. C: Left-sided tumor Shamblin class III. The arrows point to the large blood vessels, the arteria carotis interna and the arteria carotis externa. They are located outside the tumor (A), adjacent to the tumors (B), and within the tumors (C). C from Neumann et al N Engl J Med 2002;346:1459-66, with kind permission of the publisher (for complete reference please see section references).

close proximity to important blood vessels and infiltration with many smaller blood vessels. These surgeries are often technically very difficult and very time-consuming. On one hand, the surrounding blood vessels and nerves should not be damaged; on the other hand, all incoming and outgoing blood vessels of the tumors should be closed. Known complications include dramatic bleeding, damage to the cranial nerves, in particular the vagal nerve with subsequent difficulties to swallow and sore throat.

Less common are the tumors of the glomus jugulare and tympanicum (figure 21). These two structures are in such close proximity that they are sometimes called jugular-tympanic tumors. They are classified into 4 stages (stage A to D) according to the head-neck-and-ear surgeon Fisch. Examples for jugular and tympanic tumors of stages A

through D are given in Figures 21A-D. The classification helps to prepare for surgery and to compare post-operative outcomes. Patients with these tumors sometimes suffer from ear ringing at every heart beat (pulsatile tinnitus) and hearing impairment of the affected ear. The tumors are also located in close proximity to important arteries, veins, and nerves (e.g. vagal nerve and facial nerve). Surgery of these tumors will be challenging to the surgeons. Permanent damage could be caused by the tumors as well as by the surgery.



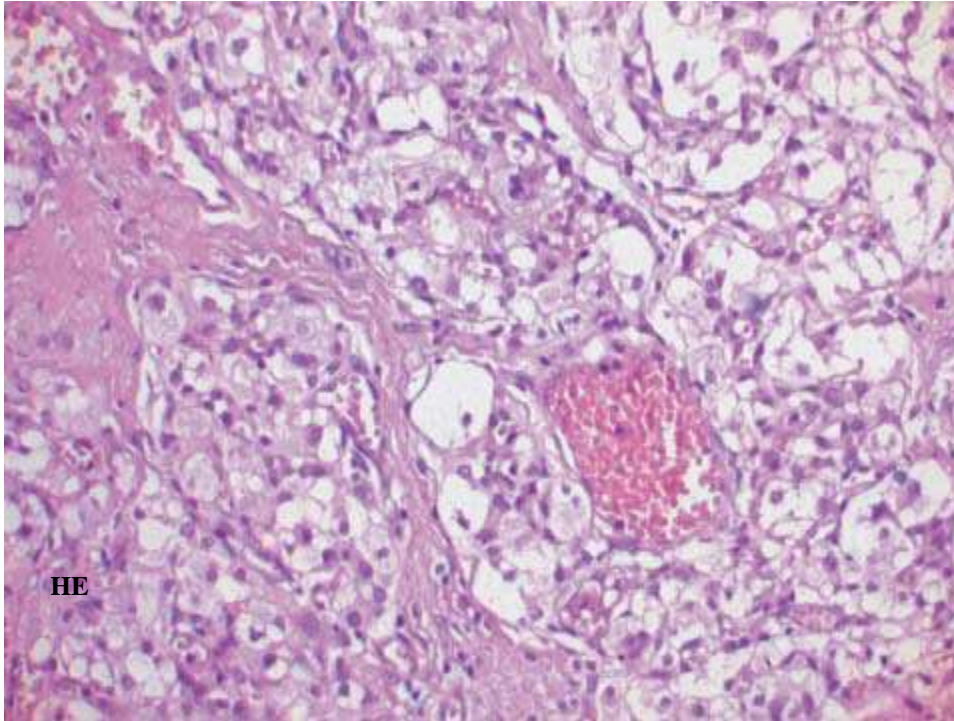
**Figure 21:** Glomus tumors of the base of the skull in the region of the petrous bone. Stages according to Fisch (stage A to D). Tumors of the stages A and B originate from the glomus tympanicum, tumors of the stages C and D originate from the glomus jugulare. A: Fisch stage A tumor of the right glomus tympanicum, CT horizontal projection at the middle ear region. B: Fisch stage B tumor of the left glomus tympanicum, CT horizontal projection at the middle ear region. C: Fisch stage C tumor of the left glomus jugulare, CT horizontal projection at the middle ear region. D: Fisch stage D tumor of the right glomus jugulare, CT horizontal projection at the middle ear region. From Offergeld et al Clinics 2012;67(S2):with kind permission of the publisher (for complete reference please see section references).

Achievements in the area of molecular genetics will have a significant impact on the treatment of glomus tumors. In addition to a better understanding of the causes of glomus tumors, the knowledge that patients with mutations in the genes SDHB, SDHC, and SDHD have a higher risk to develop glomus tumors, and the radiological examinations lead to detection of these tumors in the asymptomatic stage. It should be taken into consideration if tumors should be removed early on or if tumors with known slow progression should be removed later. Additional information is given in chapter 13, Molecular genetic diagnostic and paraganglial syndrome.

## 11. HISTOLOGY

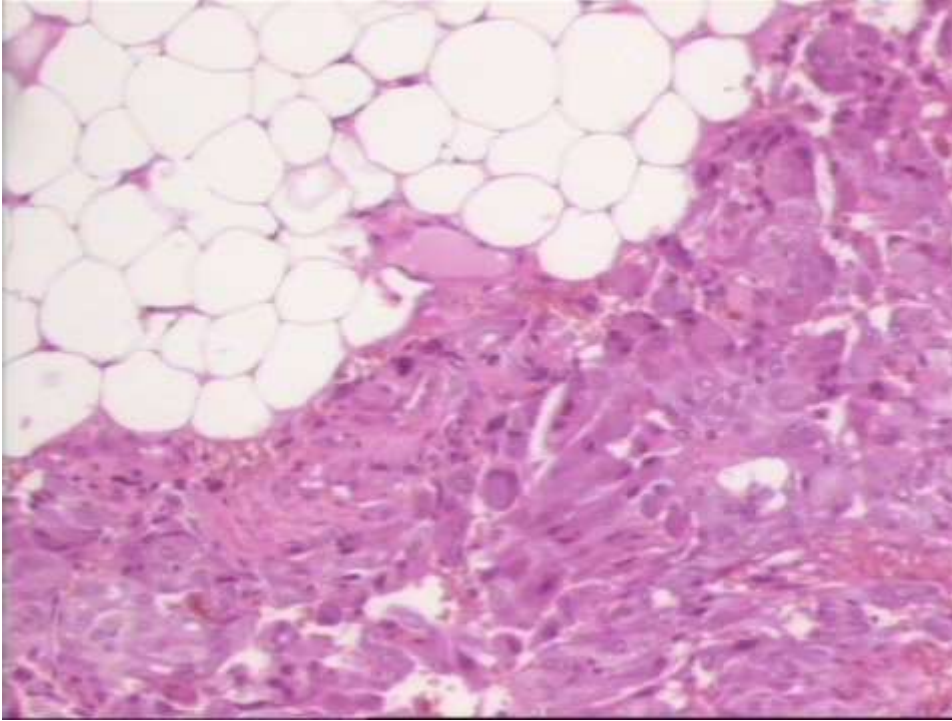
Pheochromocytoma and paraganglioma consist of chief cells and structurally supporting cells in a nested arrangement of cells (“Zellballen”). The aspect of the chief cells may be very pleomorphic, frequently with a large, prominent nucleus. These cells synthesize and store the catecholamines. The detection of chromogranin-A and synaptophysin confirms that these are endocrine tumors. The structurally supporting or sustentacular cells are slender with dendritic projections and have small nuclei. A typical characteristic of these tumors is the rich supply with small capillaries and sometimes also large blood vessels. Pheochromocytoma may have degenerative changes such as necrosis or scars made of the connective tissue.

The tumor usually grows in a nested arrangement and is well-supplied with blood vessels (Figure 22). In contrast to most other tumors, histological analysis will not be able to distinguish between benign and malignant tumors. A definitive classification as malignant tumor can only be done when metastases are detected. Metastases include spreading of tumor to lymph nodes or to other organs, so-called distal metastases, mostly in the lungs, liver, or bones.

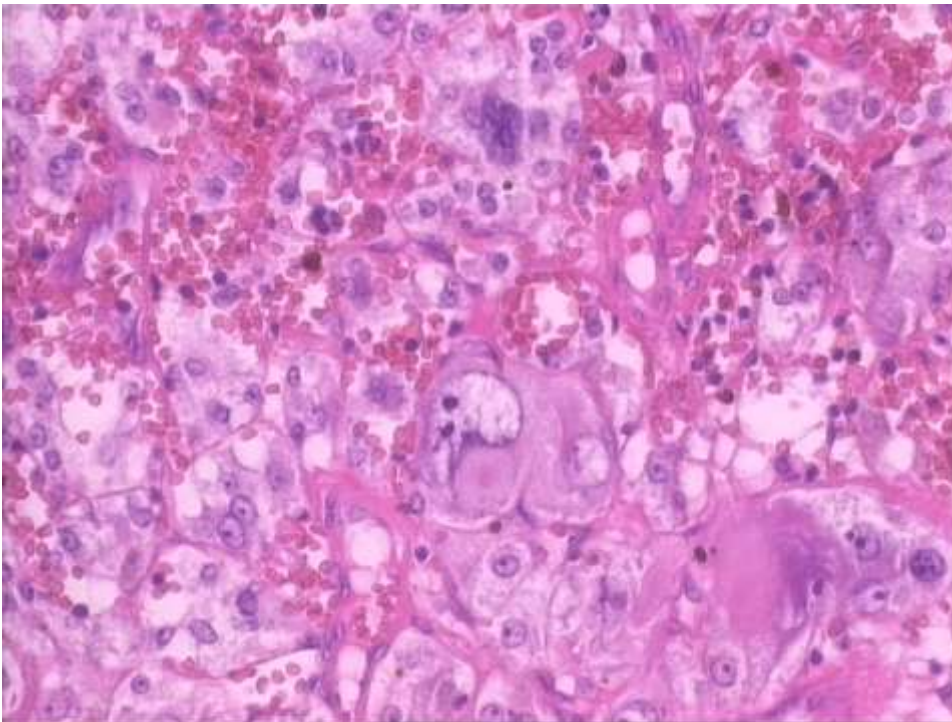


**Figure 22:** Histology of pheochromocytoma. Nested formation of the tumor is visible, adjacent to a blood vessel with dense red blood cells.

The penetration of the tumors into the surrounding fat tissue (Figure 24) may be, but is not necessarily, an indication for malignancy. Other uncertain signs of malignancy are increased increased proliferation rate, cellular pleomorphism, atypical nuclei (Figure 23), and invasion into blood vessels (Figure 25).

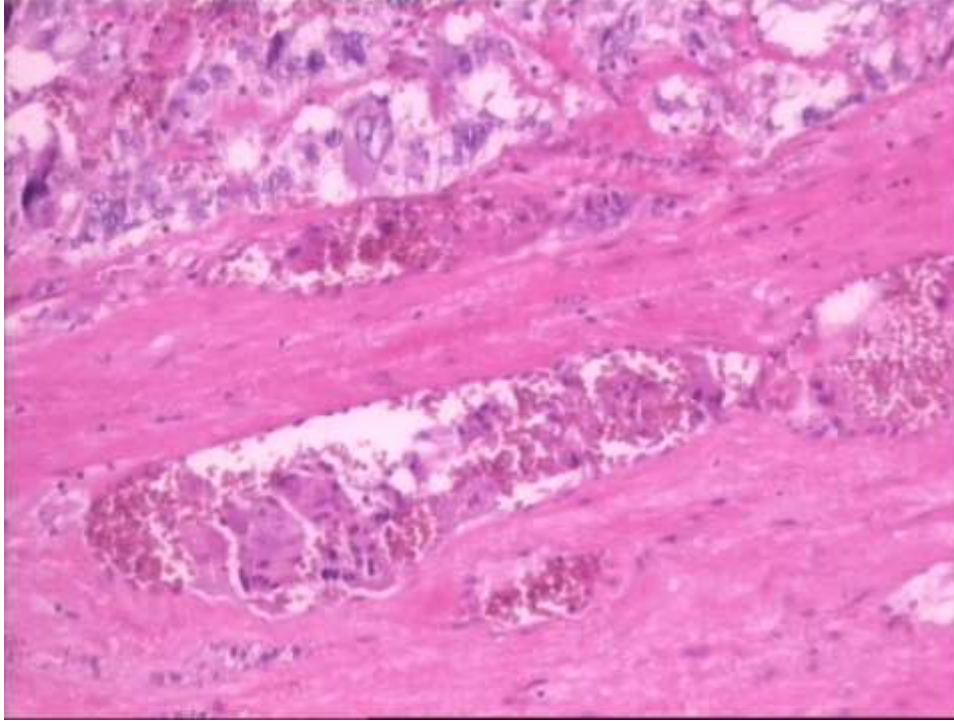


**Figure 23:** Histology of pheochromocytoma. Polymorphism of the nucleus. Tumor cells have nuclei with different sizes.



**Figure 24:** Histology of pheochromocytoma. Infiltration of the surrounding fat tissue (extra-adrenal invasion) Invasion of the tumor (right bottom of the image) into the fat tissue (left top of the image) is visible.





**Figure 25:** Histology of pheochromocytoma. Invasion of a tumor into a blood vessel. Tumor tissue (top left of the image) and tumor islets together with red blood cells in the affected blood vessel.

The histological analysis is performed by a pathologist and is sometimes scored by a point system. Most common is the scoring system of Thompson (Table 2). The scoring supports the prediction of a possible malignant development of the tumors. However, the scoring system is widely not accepted.

A non-critical use of the histological scoring system may lead to considerable uncertainty of the patients. The scoring system may only provide to a certain degree of suggestion for an adequate follow-up. This may also lead to misunderstandings when the surgeon indicates complete removal of the tumor while the pathologist may not be able to reconstruct it. In case of doubt, the expertise of the surgeon should be given more weight.



Table 2: Histomorphological scoring system for examinations of benign and malignant pheochromocytoma (PASS=Pheochromocytoma of the Adrenal gland Scaled Score)

PASS < 3; indication for a benign tumor; PASS > 4; indication for a malignant tumor (modified from Thompson, Am J Surg Pathol 2002;26: 551-566).

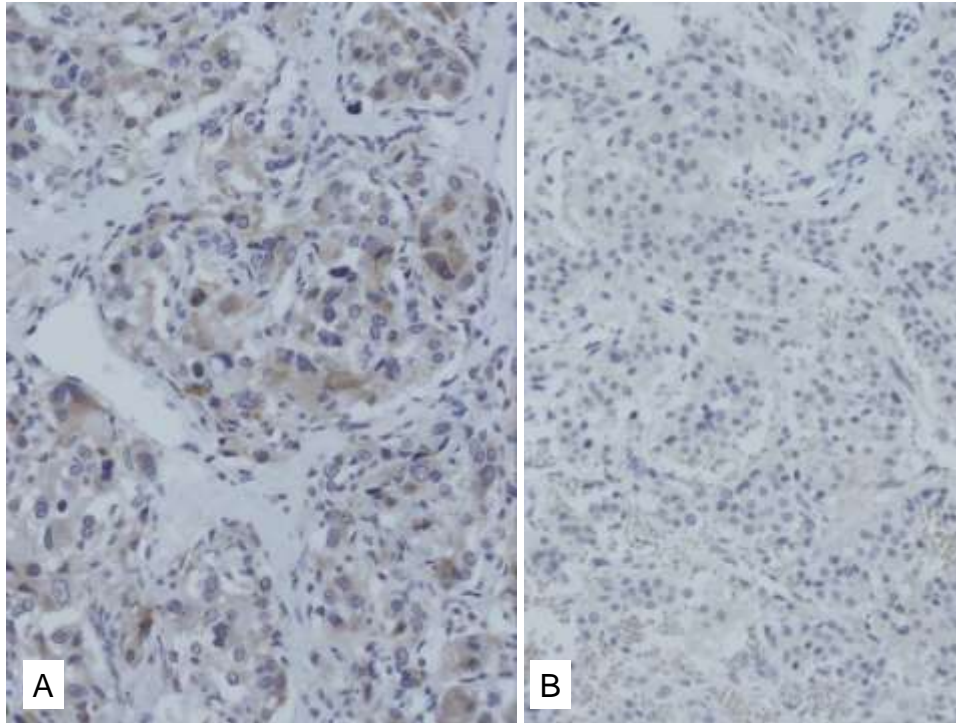
Feature	Score
<b>Diffuse growth/large cell nests</b>	2
<b>Atypical mitosis</b>	2
<b>Necrosis</b>	2
<b>Extra-adrenal invasion</b>	2
<b>High cell density</b>	2
<b>Invasion of vessels</b>	1
<b>Monotonous cell image</b>	2
<b>Capsule invasion</b>	1
<b>Spindle cells</b>	2
<b>High nuclear pleomorphy</b>	1
<b>Mitosis (&gt;3/high power field)</b>	2

### Immunohistochemistry

Immunohistochemical staining is based on antibodies for proteins. In routine histology of pheochromocytomas and paragangliomas often chromogranin-A and synaptophysin staining are used to differentiate tumor and adjacent tissue.

In the last years immunohistochemical staining has been introduced in order to obtain information, if proteins encoded by target genes show abnormal staining. If abnormal immunohistochemical staining is shown, it is likely that this protein is mis-structured due to a mutation. For example: Normally Anti-SDHB shows clearly the SDHB-SDHC-SDHD

complex by positive staining (Figure 26a). If there is no staining a mutation in one of these 3 genes has to be assumed (Figure 26b). This facilitates to select which gene should be analysed in a blood sample of the given patient.



**Figure 26:** Immunohistochemistry of a pheochromocytoma. Staining with Anti-SDHB. A Positive staining indicates that the antibody recognizes the protein. In this case it recognizes intact SDHB, SDHC and SDHD proteins. This is a “normal” finding. B Negative staining indicates changes in the corresponding protein. Here it indicates that there is likely a mutation in one of the genes SDHB, SDHC or SDHD. The patient harbored a germline mutation of the SDHB gene. From Offergeld et al Clinics 2012;67(S2): with kind permission of the publisher (for complete reference please see section references).

So far immunohistochemistry is available in addition for the proteins MEM127, SDHA and MAX, although more experience is needed to support their practical usefulness.

## 12. POSTOPERATIVE CARE

The follow-up of pheochromocytoma and glomus tumors aims to:

1. Document the success of the surgery
2. To analyze the risk for possible additional tumors by molecular genetic testing for mutations in susceptibility genes (e.g. RET, VHL, SDHA, SDHB, SDHC, SDHD, TMEM127 and MAX)
3. To discuss histological findings with the patient. In the rare event of malignant pheochromocytoma and glomus tumor, a nuclear medicine therapy or chemotherapy is to be evaluated and possibly initiated.

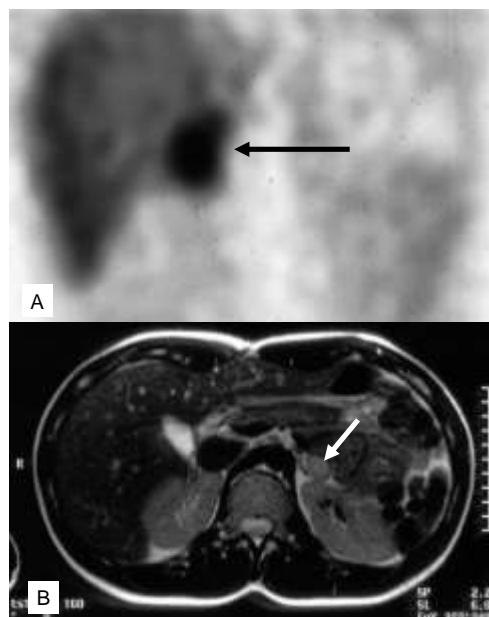
Under normal circumstances the surgeon will inform the patient that the tumor was completely removed. For this reason there is often no follow-up. The pre-operative medications are discontinued and the patient is considered as cured in most the cases. However, most patients are unsatisfied with this situation and the fact of having a rare tumor, and follow-up is advisable. Follow-up should be carried out by an endocrinologist or primary care physician or by an ear, nose, and throat doctor in the case of glomus tumors.

The blood pressure should be checked several times. Blood pressure should normalize without medication.

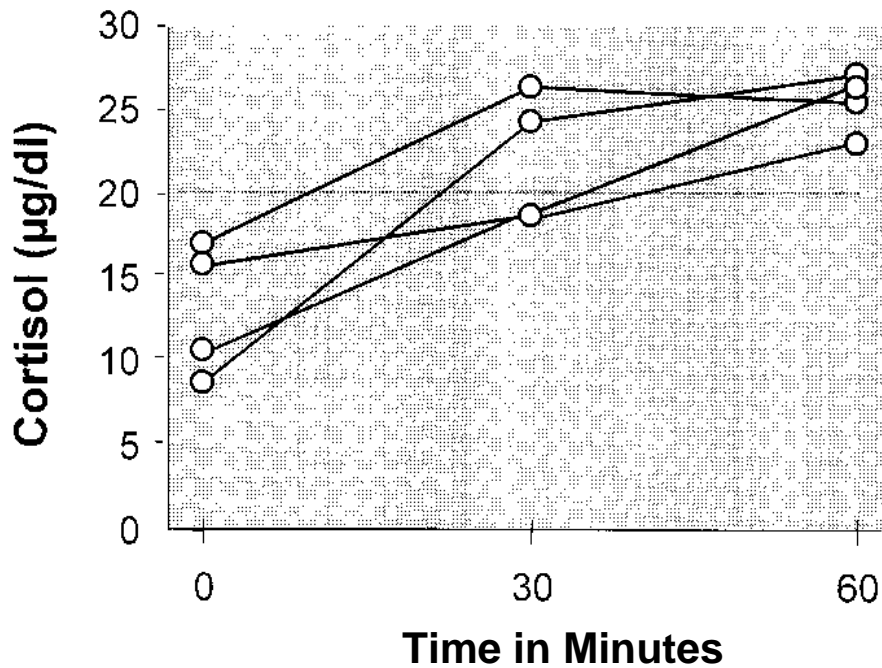
After complete removal of the tumor the elevated hormones (catecholamines and/or metanephrines) will normalize. The measurements of hormones (i.e. measurements of catecholamines and/or metanephrines) should be repeated and the decrease of these hormones to the normal range should be documented.

Post-operative imaging to confirm the complete removal of the tumor is usually not performed and not necessary when blood pressure and hormones normalize.

A special situation is the surgery of bilateral pheochromocytoma or the surgery of a tumor of the adrenal gland in patients with prior removal of a tumor at the other adrenal gland (Figure 27). In this case it is necessary to document a sufficient supply with adrenal hormones by performing a so-called ACTH test even if the patients have no symptoms. In contrast to the compensatory production of hormones of the adrenal medulla by the autonomous nervous system, the hormones of the adrenal cortex are not compensated. The ACTH test verifies the normal function of the adrenal cortex. The hormone ACTH (adrenocorticotrophic hormone) is given to the patients and elevated levels of cortisol are measured after 30 and 60 minutes. The test can be performed in an ambulatory setting (Figure 28).



**Figure 27:** 17-year-old male patient with a VHL mutation. Condition after complete removal of the right adrenal gland at 12 years of age. At the age of 17 endoscopic removal of a pheochromocytoma of the left adrenal gland (bottom) sparing sufficient adrenal gland tissue. ACTH test showed a normal cortisol increase after ACTH administration.



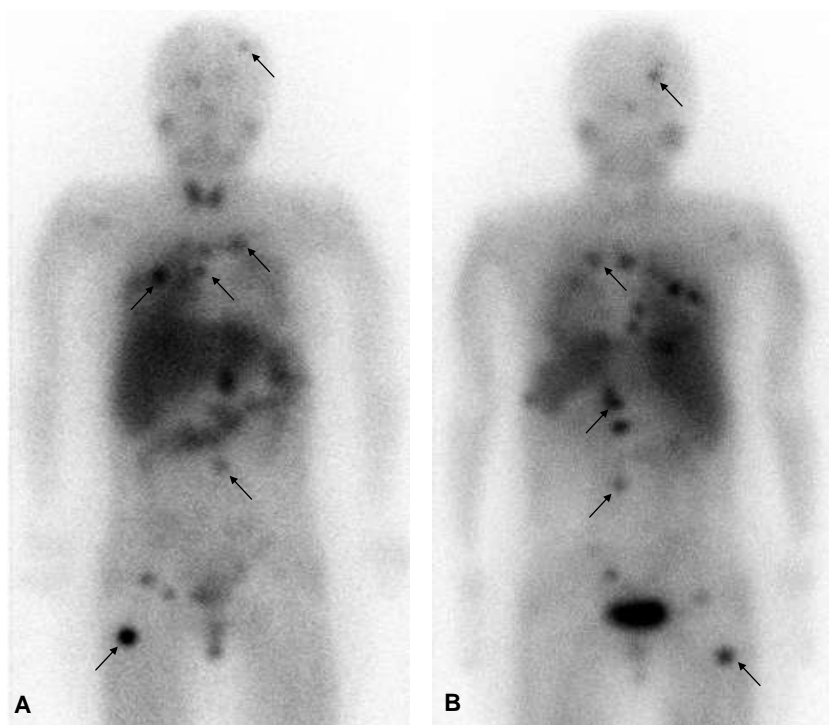
**Figure 28:** Cortisol levels of four patients after endoscopic organ-saving removal of bilateral pheochromocytoma: measurements prior and after administration of ACTH. An increase of cortisol above 20 µg/dl is expected. A significant increase after ACTH administration is shown indicating that sufficient functional adrenal gland tissue was saved. From: Neumann et al. *J Clin Endocrinol Metab* 1999;84:2608–2610 with kind permission of the publisher (for complete reference please see section references).

In case of mutations in one of the susceptibility genes, a life-long follow-up is necessary.

The precise follow-up is described in the chapters describing the respective tumors.

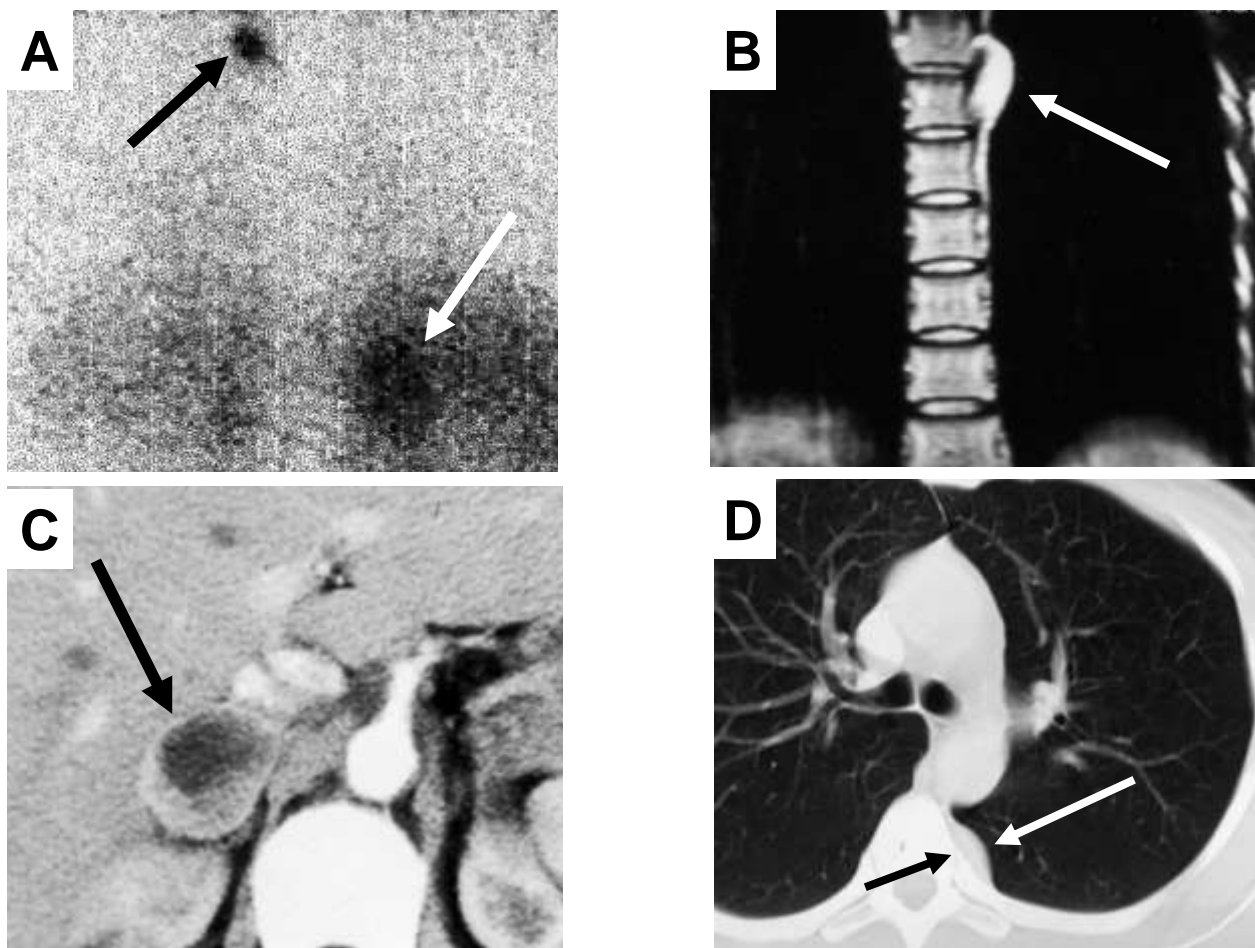
### 13. MALIGNANT PHEOCHROMOCYTOMA AND MALIGNANT GLOMUS TUMORS

Malignant pheochromocytoma and glomus tumors are treated in a similar manner to the benign tumors. Pheochromocytomas are usually benign. Only 5-10% of the pheochromocytomas are malignant and malignant glomus tumors are seemingly even rarer. The diagnosis of a malignant pheochromocytoma or glomus tumor is made when metastases are detected and confirmed by histology. Metastases are further confirmed by CT or MRI and concomitant elevated catecholamines. Even more definitive are the diagnoses utilizing  $[^{123}\text{I}]$  MIBG scintigraphy,  $[^{18}\text{F}]$  DOPA,  $[^{18}\text{F}]$  FDG  $[^{68}\text{Ga}]$  DOTATOC, or  $[^{68}\text{Ga}]$  DOTATATE-PET/CT. Metastases are usually located in the lymph nodes, the lung, the liver, or the bone (Figure 29).



**Figure 29:**  $[^{123}\text{I}]$  MIBG scintigraphy of a 16-year-old patient with malignant pheochromocytoma. The arrows point to bone metastases. A: front view. B: Rear view. The examination is the basis for a therapy with high-dose  $[^{131}\text{I}]$  MIBG.

The presence of multiple pheochromocytomas outside the adrenal glands, for instance in the abdomen, where they could be easily mistaken for lymph node metastases or the false interpretation of metastases that are indeed multiple tumors sometimes lead to the false diagnosis of a malignant pheochromocytoma (Figure 30).



**Figure 30:** False diagnosis of a malignant pheochromocytoma. [ $^{123}\text{I}$ ] MIBG scintigraphy. Rear view (A) shows a pheochromocytoma located within the adrenal gland (white arrow). The same tumor is shown in C using CT in top view. Additionally, an enrichment in the midline higher up in the thorax region was interpreted as a metastasis. This enrichment is shown in image B and C using MRI (black arrow) in front view (B) and top view (D) and is consistent with an extra-adrenal pheochromocytoma with typical location in a paraganglium. The 33-year-old female patient had a mutation of the SDHD gene. SDHD mutations frequently occur in patients with multiple tumors. From Bausch B et al. *Ann. N.Y. Acad. Sci.* 1073: 122–137 (2006)\_ 2006 New York Academy of Sciences. doi: 10.1196/annals.1353.013 with kind permission of the publisher (for complete reference please see section references).

An indication to treat is given when metastases are identified. However, if lymph node metastases have been completely removed or if histological changes in the tumors may be described as indicators for potential malignancy, there is no indication for treatment. Such patients should only be closely monitored during the follow up.

The most important treatment is surgery. All metastases should be removed if possible. Other treatment options have questionable effects.

### **Nuclear medicine treatment**

[<sup>131</sup>I] MIBG therapy is the radiation with the radioactive Iodine-131 that has been conjugated to MIBG and is used when metastases have been diagnosed by [<sup>123</sup>I] MIBG scintigraphy. The [<sup>131</sup>I] MIBG standard therapy uses a dosage of 3.7 to 11.2 GBq per therapy. There are usually several therapies required. The treatment can be repeated every two months. The team of P.A. Fitzgerald in San Francisco treats with a considerably higher dosage of 29.6 GBq. Side effect can be a severe decrease of the white blood and platelet counts (neutropenia and thrombocytopenia). This high dose MIBG therapy should therefore be combined with a preceding collection of stem cells.

[<sup>177</sup>Lu] DOTATATE, [<sup>90</sup>Y] DOTATOC, or [<sup>90</sup>Y] DOTATATE therapies are options for malignant pheochromocytoma with metastases identified by [<sup>68</sup>Ga] DOTATOC, or [<sup>68</sup>Ga] DOTATATE-PET/CT, or somatostatin receptor scintigraphy (Octreoscan). [<sup>90</sup>Y] DOTATOC, or [<sup>90</sup>Y] DOTATATE therapies use a dosage of 1.5 GBq/m<sup>2</sup> body surface or a fixed dosage of 7.4 GBq for [<sup>177</sup>Lu] DOTATATE. Usually four treatments in intervals of



two months are given. [<sup>90</sup>Y] DOTATOC, or [<sup>90</sup>Y] DOTATATE therapies may lead to kidney damage and preventive measures should be taken.

The success rate of this treatment is difficult to evaluate. Success is frequently measured as a lack of progression of the disease.

## **Chemotherapy**

Chemotherapy of the malignant pheochromocytoma is used as treatment in combination with nuclear medicine therapy or if nuclear medicine therapy was unsuccessful. The combination of cyclophosphamide, vincristine, and dacarbazine (so called Averbuch protocol) (CVD) is the standard chemotherapy for malignant pheochromocytoma. The 2-day treatment is repeated 3 to 6 times in intervals of one month dependent on the response and tolerance of treatment. The success of the treatment is measured by decreased plasma or urine catecholamine levels and the reduction of tumor size. A complete remission is seen in 20 % of the patients and partial remission in 45 % of patients.

After unsuccessful CVD therapy other substances such as Vindesin/DTIC, AraC, CTD plus Anthracycline, combinations of Vepesid, Caboplatin, Vincristin, Cyclophosphamide, Adriamycin, or Temozolomide plus Thalidomide may be used.

Novel, experimental therapies include treatment with HSP-90 and hTERT inhibitors, Lomustin, Capecitabin, Thalidomide, Lenalidomide, or Sunitinib, Sorafenib,

Temsirolimus, Bevacizumab, and combinations of them. The most favorite treatment is currently Sunitinib.

### **Preservation of patients (autologous) stem cells**

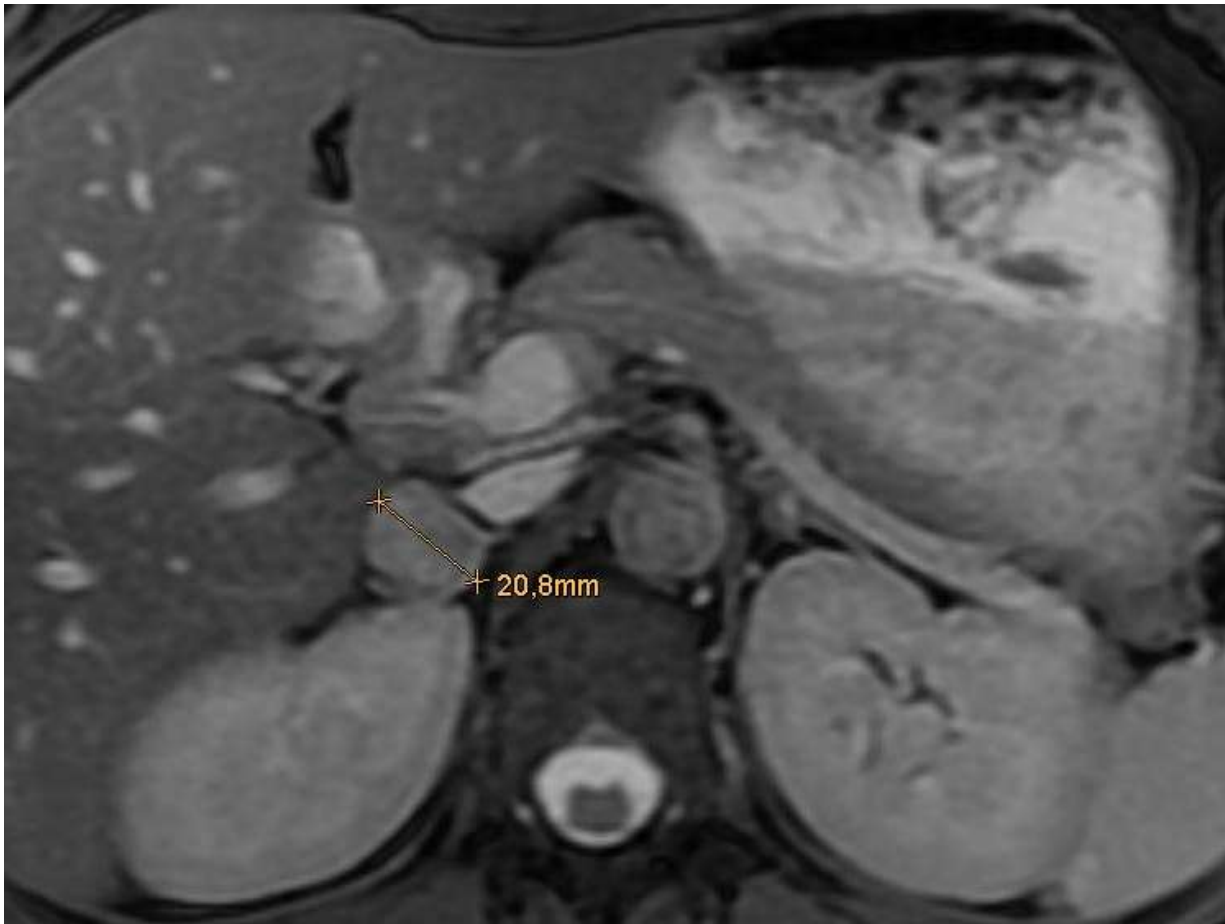
It is recommended to perform a stem cell apheresis prior to a scheduled chemotherapy or high dose MIBG therapy. It serves as preservation of the patients' own (autologous) stem cells in case of a drop in the numbers of immune cell (aplasia) after chemotherapy or MIBG therapy. This is particularly relevant for an existing infiltration of tumor cells into the bone marrow. However, the preservation of stem cells from these patients is very difficult. The collection of stem cells is usually preceded by stimulation with G-CSF (Neupogen or Granocyte). G-CSF is administered via daily injections (for several days) underneath the skin. Mobilization of stem cells using Cyclophosphamide is nowadays used only in exceptional cases.

## 14. EXCEPTIONAL SITUATIONS

### *Pheochromocytoma during pregnancy*

The occurrence of pheochromocytoma during pregnancy is extremely rare but is a dangerous situation. There are several cases reported in the literature and the Freiburg International Registry. If left undiagnosed and untreated, the course of the disease during pregnancy can be life-threatening.

There are not many well documented data on patients with pheochromocytoma during pregnancy. Figure 63 shows a pheochromocytoma of 2.5 x 2.0 cm in diameter which was silent during pregnancy, the blood pressure was well documented, until the 38<sup>th</sup> week of gestation. The patient had severe hypertension and was diagnosed with preeclampsia. She had immediately cesarean section, and the child was healthy. Postoperative investigations included protein excretion in the urine which was normal, unusual in preeclampsia. Then under the suspicion of renal artery stenosis, another potential reason of hypertension, an ultrasonography (Duplex) of the kidneys was performed and showed as an "incidental" finding the tumor in the right adrenal. Metanephrines were elevated and MIBG scintigraphy positive.



**Figure 63:** A patient with pheochromocytoma and pregnancy. The tumor is 2.0 x 2.5 cm in diameter. The patient had normal blood pressure frequently documented, and not before the 38<sup>th</sup> week of gestation the patient had markedly elevated blood pressure.

Another case was reported in 1979. The 22-year-old patient complained of ailments for 6 months. The patient presented with severe headaches and severe hot flashes. The blood pressure was considerably elevated (280/120 mmHg). Cesarean section and removal of the pheochromocytoma took place during the 9<sup>th</sup> month of the pregnancy. Both, mother and baby survived safe and sound. The mother was years later diagnosed with von Hippel-Lindau disease which was the cause of the pheochromocytoma.

An early and correct interpretation of the symptoms and findings are critical for the timely diagnosis of pheochromocytoma during pregnancy. The previously dangerous surgery can meanwhile be done endoscopically with low risk for mother and baby, preferentially during the 2<sup>nd</sup> trimester of the pregnancy. The presurgical medical treatment to prevent cardiovascular complications during surgery is essentially similar as in non-pregnant patients.

#### *Pheochromocytoma in children and adolescents*

The occurrence of pheochromocytoma in children and adolescents particularly raises the question of the etiology (cause of the disease). A pheochromocytoma can develop at early ages as mentioned for the several pheochromocytoma-associated syndromes (Chapters 14-17). The age that a pheochromocytoma is diagnosed is therefore much younger than that for patients with sporadic pheochromocytoma. The analysis of the data in the Freiburg International Registry has demonstrated that pheochromocytomas are associated to these syndromes in children (4-10 years) in 90% and in adolescents (11-18 years) in 70 % of the cases. Mutations can be detected in the majority of the patients with pheochromocytoma in these age groups. The most frequent mutations are located in the VHL gene.

## **15. MOLECULAR GENETIC DIAGNOSIS**

The molecular or molecular genetic diagnostics aim to detect inherited diseases. Detection of inherited pheochromocytoma or glomus tumors allow for appropriate prevention and follow-up. Patients who are carrier of specific mutations carry a higher risk for a particular clinical outcome of the disease depending on the mutated gene: age of appearance of tumors, location of tumors, multiplicity of tumors, benignity and malignancy of tumors for tumors within the autonomous system (i.e. paraganglial tumors) and outside this system for instance tumors of the thyroid, skin, eye, central nervous system, kidney, and pancreas.

The “classical” diseases that form a group together with inherited pheochromocytoma and glomus tumors are: Multiple endocrine neoplasia type 2, von Hippel-Lindau disease, neurofibromatosis type 1, and the paraganglial syndrome type 1 to 4. These diseases are summarized in table 3 with their respective characteristics. A more detailed description is given in chapters 14 to 17.

### **Molecular genetic analysis**

The molecular genetic analysis uses similar principles. A blood sample will be used for the analysis of the genetic material (i.e. DNA). Depending on the gene of interest, one or several small fragments within the coding region (exons) will be enriched using a special method (PCR) and will be further analyzed. These fragments will be sequenced. Since sequencing is rather expensive other methods are used to determine if the gene

Table 3. Inherited Diseases with pheochromocytoma and glomus tumors

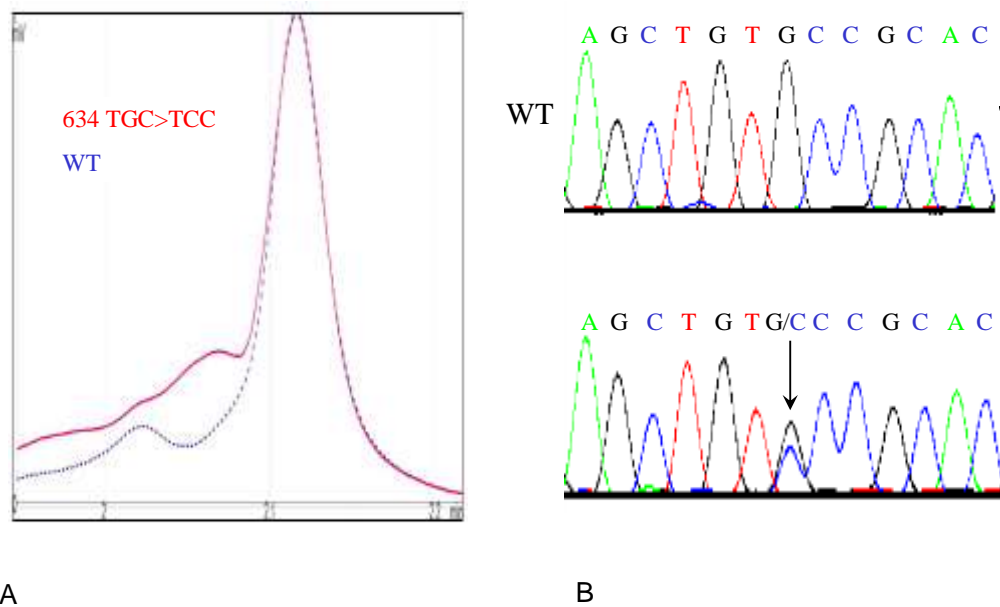
	<b>MEN 2</b>	<b>VHL</b>	<b>NF 1</b>	<b>PGL1</b>	<b>PGL3</b>	<b>PGL4</b>
Average age at time of diagnosis	<30 years	30 years	42 years	32 years	41 years	31 years
single/multiple tumors	33% / 67%	42% / 58%	83% / 17%	26 / 74%	89% / 11%	72% / 28%
Localization in adrenal glands; extra-adrenal in the posterior abdomen	Almost exclusively in adrenal glands	88% / 12%	94% / 6%	53% / 21%	Very rare	28% / 50%
Thoracic pheochromocytoma	Extremely rare	Rare	Very rare	18%	Very rare	9%
Glomus tumors	Very rare	Very rare	Very rare	79%	100 %	31%
Malignancy	4%	Rare	12%	Rare	Never been observed	35%
Other tumors	Medullary thyroid carcinoma, Hyperparathyroidism	Retinal Angioma, Hemangioblastoma of the central nervous system, kidney carcinoma, Islet cell tumors	Neurofibroma, Iris Hamatoma, Nerve sheath tumors	None	None	Kidney carcinoma (rare)
Inheritability	autosomal-dominant*	autosomal-dominant	autosomal-dominant	autosomal-dominant	autosomal-dominant	autosomal-dominant
Name of the genes	<i>RET</i>	<i>VHL</i>	<i>NF1</i>	SDHD	<i>SDHC</i>	<i>SDHB</i>
Chromosomal localization of the genes	10q11.2	3p25-26	17q11.2	11q23	1q21	1p36
Number of exons	21	3	60	4	6	8

\*only applies to children from male carriers

adapted from Bausch et al. N Engl J Med 2006

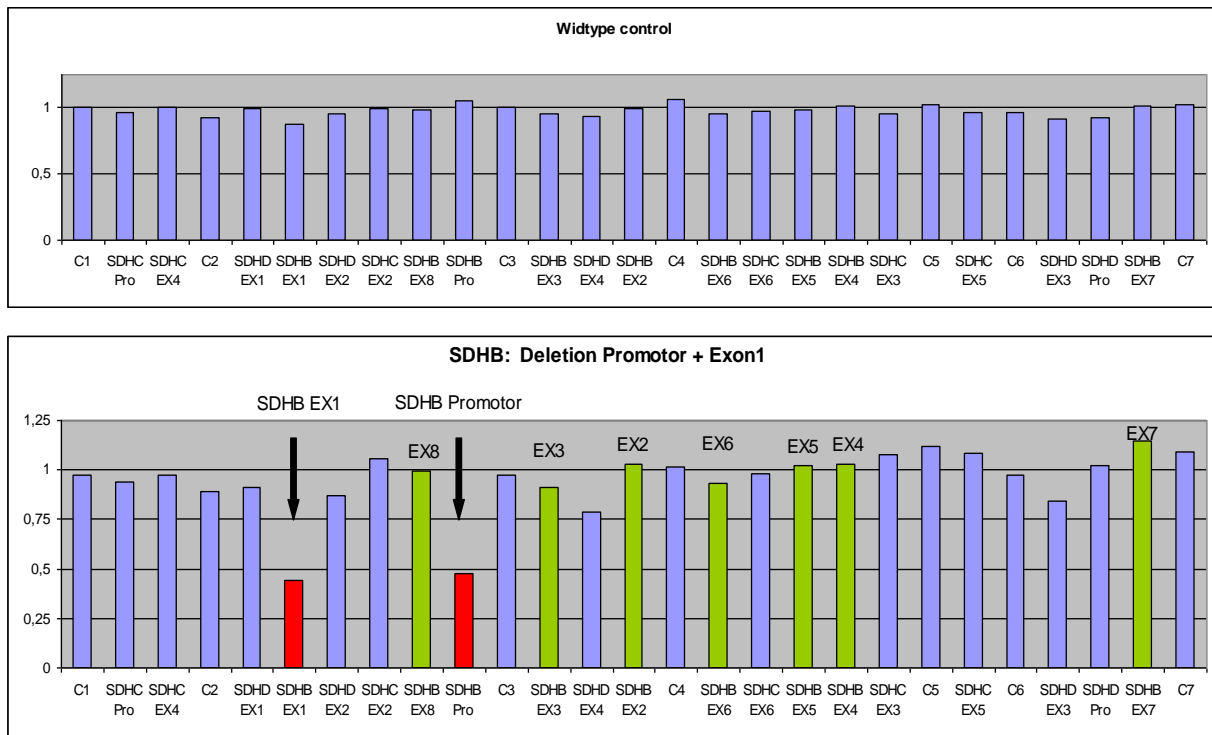
of interest contains a mutation or polymorphism or not. The so-called method of DHPLC (Denaturing High-Performance Liquid Chromatography) is used that acquires a curve (chromatography) with either a wild-type (normal chromatogram profile) or aberrant profiles (Figure 31). For the detection of large deletions in a gene (i.e. one or several

exons) the methods MLPA (multiple ligation-dependent probe amplification) (Figure 32) or QMPSF (quantitative multiplex PCR of short fluorescent fragments) are used. All the mutations in the genes discussed here are summarized as a table in Chapter 22.



**Figure 31:** Chromatography (so-called DHPLC method) and sequencing. A: DHPLC. A clear difference between the red curve and the dotted normal curve can be seen. B: The corresponding sequencing with normal findings (WT= wild-type) at the top and at the bottom a double peak in blue (C= cytosine) and black (G= guanine) (arrow). From Neumann et al. *N Engl J Med* 2007;357:1311-5, with kind permission of the publisher (for complete reference please see section references).





**Figure 32:** Confirmation of a large deletion of the SDHB gene using the MLPA (multiple ligation-dependent probe amplification) method. Top: normal findings. Bottom: mutation. It is expected that one gene or exon out of the two genes within the analyzed region is missing. The decrease of the height of the bar by half indicates a mutation. This graph shows mutations for SDHB exon 1 (SDHB Ex 1) and the preceding promoter (SDHB promoter) (red bars, arrows). The other exons of the SDHB gene are green and reach a height of 1 (= 100 %).

## Structure and analysis of the candidate genes

The MAX gene

The MAX gene is a new gene identified in patients with hereditary paraganglial tumors.

The MAX gene contains 5 exons. So far, mutations of the MAX gene have been found

in patients at an age younger than 30 years at diagnosis with uni- or bilateral adrenal pheochromocytomas. Evidence for preferential paternal transmission of *MAX* mutations has been found among affected carriers. This means that mutation carriers will have tumors only if the mutation is inherited from the father. The available data are so far limited and more information is needed to determine when to look for mutations in this gene.

#### The NF1 gene

The NF1 gene is one of the largest genes. It consists of 60 exons. Mutations associated with pheochromocytoma have been described which are spread over the entire gene. In addition large deletions of the NF1 gene associated with pheochromocytoma have been reported. Important is that all patients with pheochromocytoma and a mutation of the NF1 gene had also cutaneous manifestations of neurofibromatosis type 1. Therefore, mutation analyses – which are very expensive – of the NF1 gene are not recommended.

#### The RET gene

The RET gene is analyzed to detect mutations that predispose for multiple endocrine neoplasia type 2 (MEN 2). The disease is described in Chapter 14. The RET gene should be analyzed when the patient or a family member was diagnosed with medullary

thyroid carcinoma. However, the family history is not always a good indication and the pheochromocytoma might be the first symptom.

The RET gene consist of 21 exons. Almost all patients with MEN 2 have mutations of the RET gene. These mutations are only present in a few out of the 21 exons and these exons should be analyzed. Some of these few exons are mutated very frequently, some of these exons are rarely mutated, and others are only mutated in a few cases.

A complete list of the RET mutations can be found on the internet: [http://arup.utah.edu/database/MEN2/MEN2\\_display.php?sort=1#m](http://arup.utah.edu/database/MEN2/MEN2_display.php?sort=1#m).

Most mutations (75 % of patients with MEN 2) are located in codon 634 in exon 11. Rarer are mutations in codons 609, 611, 618, and 620, all located in exon 10. The severe form of MEN 2, also designated MEN 2B, is associated with aggressive disease progression and extreme height and is usually characterized by mutations in the codon 918 located in exon 16. Pheochromocytomas are only observed in 50 % of patients with MEN 2 and are only observed in association with mutation in exons 10, 11, and 16. We only observed one case of a mutation in exon 13 in our entire patient cohort of almost 2000 patients with pheochromocytoma or glomus tumors.

Deletions of large fragments (i.e. one or several exons) have not been described for MEN 2. A special analysis for these large deletions is not necessary. The analysis of the RET gene utilizes sequencing.

Almost all patients with MEN 2 develop a medullary thyroid carcinoma that can be identified by elevated calcitonin levels in the blood. Since the majority of the patients with MEN 2 develop pheochromocytoma as adults (i.e. at a time point when a medullary

thyroid carcinoma should be already present with high probability), normal calcitonin levels make the presence of MEN2 unlikely.

#### The SDHA gene

The SDHA gene is a new gene identified in patients with hereditary paraganglial tumors. The SDHA gene contains 15 exons making the analysis time consuming and expensive. So far, mutations of the SDHA gene have been found in patients under an age at diagnosis of less than 30 years, patients with multiple tumors, extraadrenal tumors and malignant tumors. The available data are so far limited and more information is needed for a policy when to look for mutations in this gene.

#### The SDHB gene

The SDHB gene is analyzed to identify patients with paraganglial syndrome type 4 (described in Chapter 14). Patients with mutations in the SDHB gene may develop pheochromocytoma of the adrenal glands, extra-adrenal pheochromocytoma in the abdomen, pelvis, and thorax, as well as glomus tumors. Mutations of the SDHB gene very rarely lead to tumors in other organs. One example is carcinoma of the kidney, however, they are much less common than in von Hippel-Lindau disease.

The SDHB gene consists of 8 exons and codes for a protein (SDHB) of 280 amino acids. Mutations might appear in all 280 codons. A list of all described mutations is

available on the internet:

[http://chromium.liacs.nl/lovd\\_sdh/variants.php?action=search\\_unique&select\\_db=SDHB](http://chromium.liacs.nl/lovd_sdh/variants.php?action=search_unique&select_db=SDHB)

Selected mutations of the SDHC gene that were identified in our laboratory in Freiburg are listed in Chapter 22.

The SDHC gene

The SDHC gene is analyzed to identify patients with paraganglial syndrome type 3 (described in chapter 17). Patients with mutations in the SDHC gene mostly only develop glomus tumors. Mutations of the SDHC gene are very rare in patients with pheochromocytoma of the adrenal glands, extra-adrenal pheochromocytoma of the abdomen, or thorax. The analysis of the SDHC may be restricted to patients with glomus tumors.

The SDHC gene consists of 6 exons and codes for a protein (SDHC) of 169 amino acids. Mutations might appear in all 169 codons. A list of all described mutations is available on the internet:

[http://chromium.liacs.nl/lovd\\_sdh/variants.php?action=search\\_unique&select\\_db=SDHC](http://chromium.liacs.nl/lovd_sdh/variants.php?action=search_unique&select_db=SDHC)

. Selected mutations of the SDHC gene that were identified in our laboratory in Freiburg are listed in Chapter 22.

The SDHD gene

The SDHD gene is analyzed to identify patients with paraganglial syndrome type 1 (described in chapter 17). Patients with mutations in the SDHD gene may develop

pheochromocytoma of the adrenal glands, extra-adrenal pheochromocytoma of the abdomen, pelvis, or thorax, as well as glomus tumors. Frequently, patients with mutations of the SDHD gene have more than one tumor.

The SDHD gene consists of 4 exons and codes for a protein (SDHD) of 160 amino acids. Mutations might appear in all 160 codons. A list of all described mutations is available on the internet: [http://chromium.liacs.nl/lovd\\_sdh/variants.php?action=search\\_unique&select\\_db=SDHD](http://chromium.liacs.nl/lovd_sdh/variants.php?action=search_unique&select_db=SDHD). Selected mutations of the SDHC gene that were identified in our laboratory in Freiburg are listed in Chapter 22.

#### The SDHAF2 (SDH5) gene

Mutations of the SDHAF2 gene have been identified very recently in patients with glomus tumors. The associated syndrome is the paraganglial syndrome type 2 (described in chapter 17). So far only two families world-wide have been described with mutations in this gene. Only patients with glomus tumors and a family history of glomus tumors should be screened for the SDHAF2 gene. Only patients have been described who inherited the mutation from the father.

The SDHAF2 gene consists of 4 exons and codes for a protein (SDHAF2) of 167 amino acids. Although a large patient population of patients with glomus tumors was screened, only one mutation in the SDHAF2 gene has been described.

## The TMEM127 gene

The TMEM127 gene is also a new gene identified in patients with hereditary paraganglial tumors. The TMEM127 gene contains 3 exons in which the mutations are spread over 239 codons. So far, mutations of the TMEM127 gene have been found mainly in patients younger than 42 years at diagnosis, patients with multiple tumors, extraadrenal tumors and sometimes malignant tumors. The available data are so far limited and more information is needed to determine when to look for mutations in this gene.

## The VHL gene

The VHL gene is analyzed to identify patients with von Hippel-Lindau Disease. This disease is described in chapter 15. Patients with pheochromocytoma who also suffer from hemangioblastoma, angioma of the retina, or hemangioblastoma of the nervous system either themselves or who have a family member with those conditions should be screened. These tumors lead to vision impairment in one or less commonly in both eyes and patients should be asked about vision impairments. Tumors of the nervous system are mostly located in the cerebellum, in the spinal marrow or in the spinal cord. Patients with von Hippel-Lindau disease may also develop kidney carcinoma and a family history of these tumors might be an important indication. However, pheochromocytoma is not infrequently the first indication of von Hippel-Lindau disease.

The VHL gene consists of 3 exons and codes for a protein (pVHL) of 213 amino acids. Mutations have only been reported in amino acids 54 to 213 (i.e. codons 54 to 213).

The designation of the nucleotides changed over time. The new designation subtracts 213 nucleotides. The old designation for the Schwarzwald mutation VHL 505 T>C is now designated as 292 T>C (p.Y98H). A list of the described mutation can be found on the internet: <http://www.umd.be/VHL/>.

### **When should patients be screened for mutations?**

Which gene should be analyzed?

The answers to these questions are based on the results obtained from our research project on pheochromocytoma and glomus tumors sponsored by the German Cancer Aid (Deutsche Krebshilfe). All results are based on the International Pheochromocytoma and Glomus Tumors Registry (based in Freiburg, Germany). The majority of the patients (approximately 950) live in Germany.

Patients with pheochromocytoma have a risk of 20 to 30% to carry one mutation and patients with glomus tumors have a risk of approximately 27%. Based on these data, the question can be raised when a genetic analysis should be conducted and which gene(s) should be analyzed. The relative risks for patients are high and for a long time it was common practice to offer all patients a genetic analysis. However, costs for these tests should be taken into consideration.

### **Pheochromocytoma—important details from the patients history**



Prior to a genetic analysis specific information should be collected to identify possible gene(s) of interest. The age at diagnosis of patients with pheochromocytoma due to a germline mutation is considerable younger compared to patients with sporadic pheochromocytoma. There is no clear age limit but an age younger than 30 to 45 years might indicate a mutation.

Additional diseases should be taken into consideration as well. After surgery of a medullary thyroid carcinoma the genetic analysis could be limited to the analysis of the RET gene; angioma of the eye or the central nervous system warrants an analysis of the VHL gene. In case of kidney carcinomas the VHL gene should be analyzed first and then the SDHB gene. Patients with pheochromocytoma and glomus tumors should be analyzed for mutation in the SDHD and SDHB genes. Patients with neurofibroma and other indications for neurofibromatosis type 1 do not require genetic analysis. It is almost certain that these patients carry a mutation of the NF1 gene.

A family history or pedigree analysis should be conducted. Special emphasis should be given to the diseases mentioned above. This analysis might give an indication of genes with high probability of mutations.

Patients with young age (younger than 45 years at diagnosis), a family history, multiple pheochromocytoma, extra-adrenal pheochromocytoma, pheochromocytoma of the thorax, and patients with malignant pheochromocytoma have a distribution of mutations as depicted in figures 33 to 38. The scientific literature shows algorithms for suggestion of genetic testing which are somewhat different from author to author.

## **Glomus tumors—important details from the patients history**

Patients with glomus tumors can be limited to the analysis of the SDHB, SDHC, and SDHD genes. Glomus tumors appear unfrequently in conjunction with MEN2, VHL disease, and NF1. However, they only appear after the manifestation of the typical lesion for the respective disease. The analysis of the RET, VHL, and NF1 genes are therefore not indicated in patients with glomus tumors, unless these patients have additional lesions. The young age (<40 years), multiple glomus tumors, concurrent pheochromocytoma, and malignancy of the glomus tumor and/or a family history for pheochromocytomas or glomus tumors are helpful indications to select the most likely gene(s) for mutation analysis: The SDHB gene is often mutated in single extraadrenal, not infrequently malignant tumors, SDHC typically in single benign glomus tumors, and SDHD frequently in benign, multiple adrenal and extraadrenal pheochromocytomas and glomus tumors.

## **Summary for patients with single, benign pheochromocytoma located within the adrenal glands**

**All genes:** mutations are very unlikely in patients over 30 years of age at time of diagnosis of pheochromocytoma unless there are further indications in family history, tumor location, numbers of tumors, or malignancy.

**MAX gene:** Limited informations are available showing only adrenal tumors.

**NF1 gene:** All patients exhibit skin and eye symptoms of a NF1. A genetic analysis of the NF 1 gene is not necessary.

**RET gene:** All patients with mutations in the RET gene were diagnosed with medullary thyroid carcinoma. These patients also have elevated blood calcitonin levels. Mutations have only been detected in exons 10, 11, 13, and 16. Analysis of the RET gene is therefore reasonable when calcitonin levels are elevated or a medullary thyroid carcinoma has been diagnosed.

**SDHA gene:** Published data are scarce and publications showing the pattern of the disease are pending.

**SDHB gene:** A family history for pheochromocytoma or glomus tumors is rare. Multiple tumors or a glomus tumor are rare. The genetic analysis is reasonable.

**SDHC gene:** Tumors located in the adrenal glands are very rare. Genetic analysis is not reasonable.

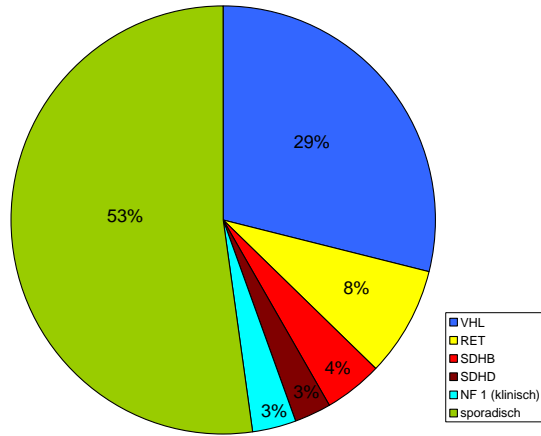
**SDHD gene:** Approximately 50% of the patients have glomus tumors. Another 50 % of the patients have a family history for pheochromocytoma or glomus tumors. The genetic analysis is reasonable in particular, if pheochromocytoma or glomus tumor is known to be present in the father.

**SDHAF2 gene:** Tumors located in the adrenal glands have not been described. Genetic analysis is not reasonable.

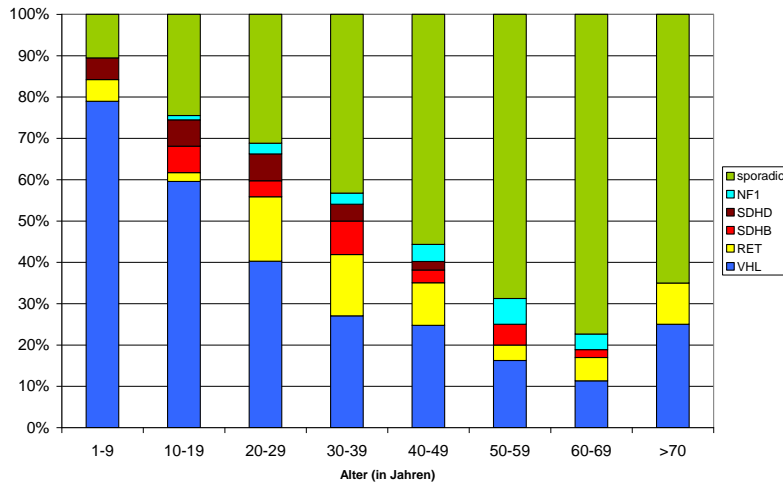
**VHL gene:** Approximately one third of the patients have retinal angioma or a hemangioblastoma of the nervous system. Another third of the patients have a family history for tumors related to VHL. The analysis of the VHL gene is reasonable.

**TMEM 127 gene:** So far there is only one report about the clinical picture. The genetic analysis may be useful.

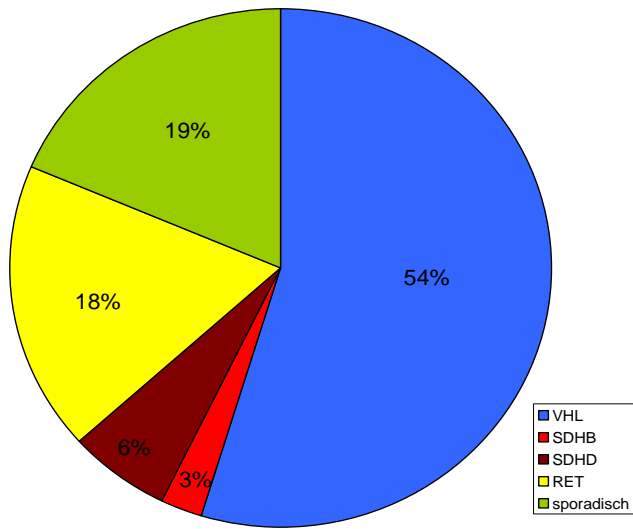
The results of the genetic analysis for one-sided, benign tumors of the adrenal glands are summarized in Figures 43 and 44. It is easy to recognize that patients older than 40 years of age rarely have mutations in the candidate genes, taking into consideration a thorough family history and summary of the important clinical findings (status of the skin, calcitonin level).



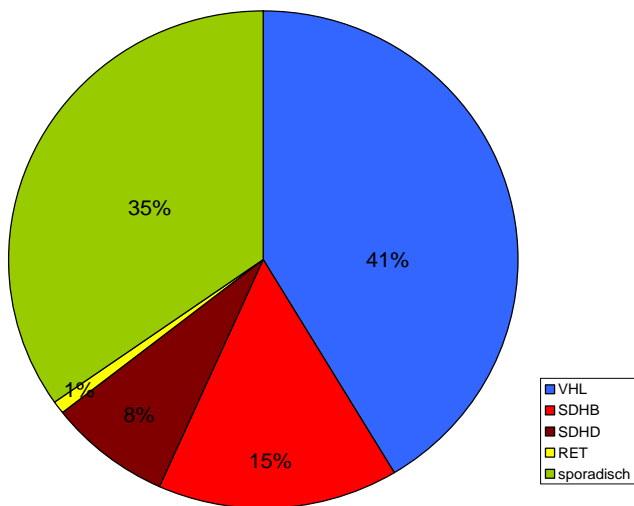
**Figure 33:** Distribution of mutations in 698 patients with pheochromocytoma.



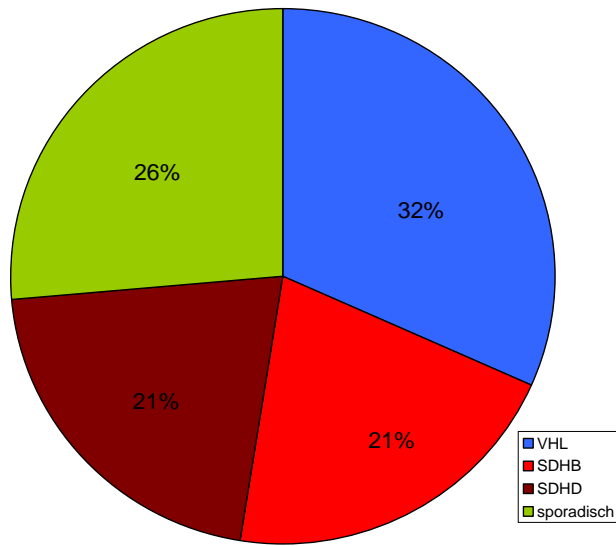
**Figure 34:** Distribution of mutations in 698 patients with pheochromocytoma. Patients are presented in decades, e.g. 1-9 years, 10-19 years ect., are summarized as 100 %. The color code demonstrates how many patients develop sporadic tumors or develop tumors based on mutations in the given genes.



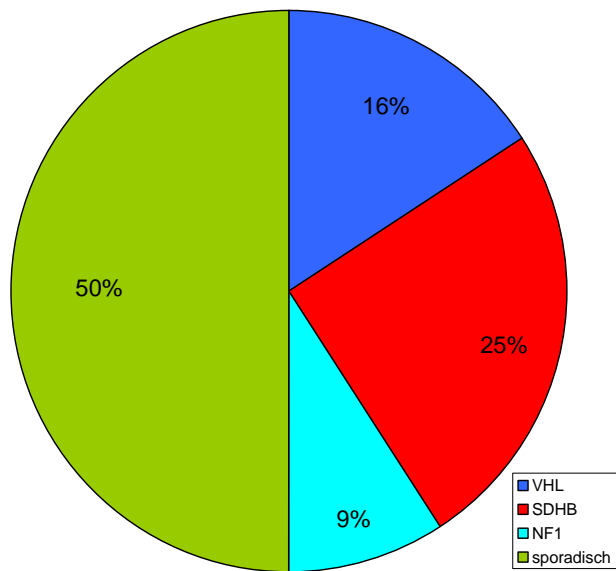
**Figure 35:** Distribution of mutations in patients with multiple pheochromocytoma.



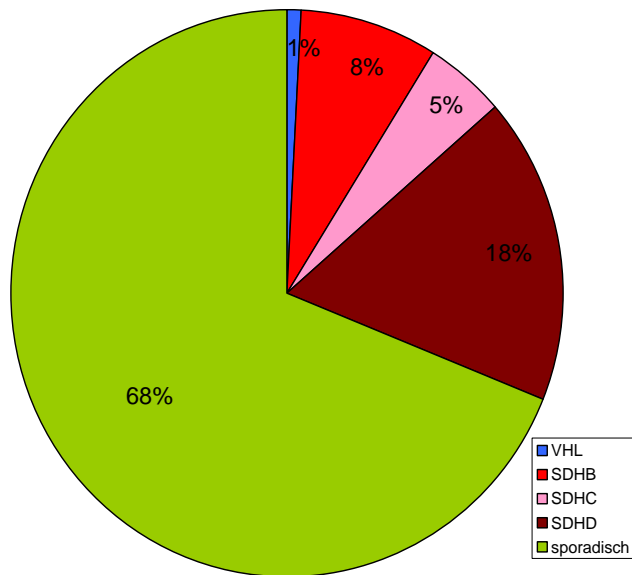
**Figure 36:** Distribution of mutations in patients with extra-adrenal abdominal pheochromocytoma.



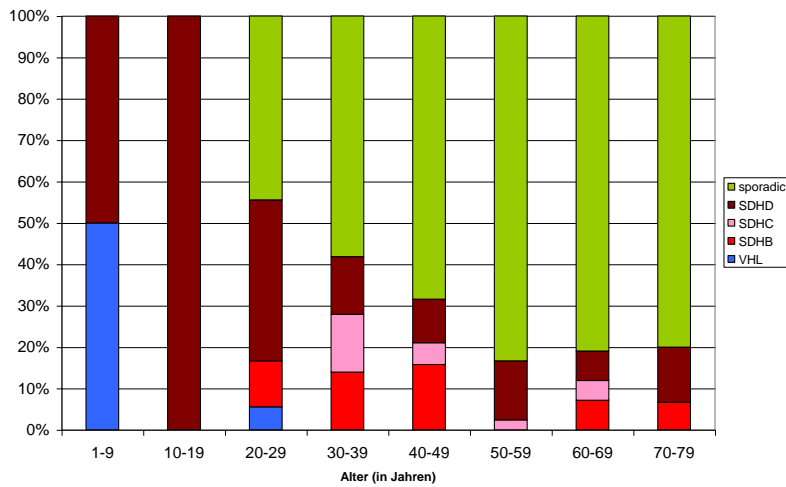
**Figure 37:** Distribution of mutations in patients with thoracic pheochromocytoma.



**Figure 38:** Distribution of mutations in patients with malignant pheochromocytoma.

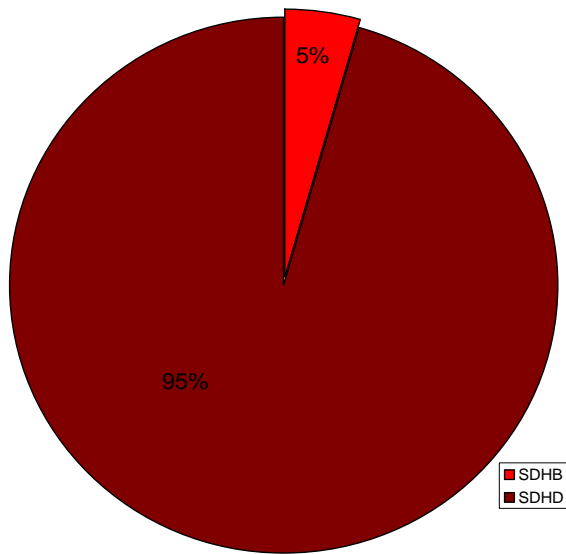


**Figure 39:** Distribution of mutations in 259 patients with glomus tumors.

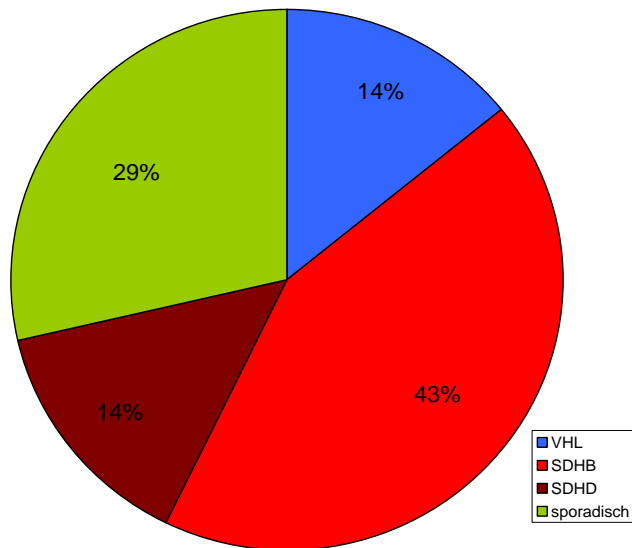


**Figure 40:** Distribution of mutations in patients with glomus tumors. Patients are presented in decades, e.g. 1-9 years, 10-19 years ect., are summarized as 100 %. The color code demonstrates how many patients develop sporadic tumors or develop tumors based on mutations in the given genes.

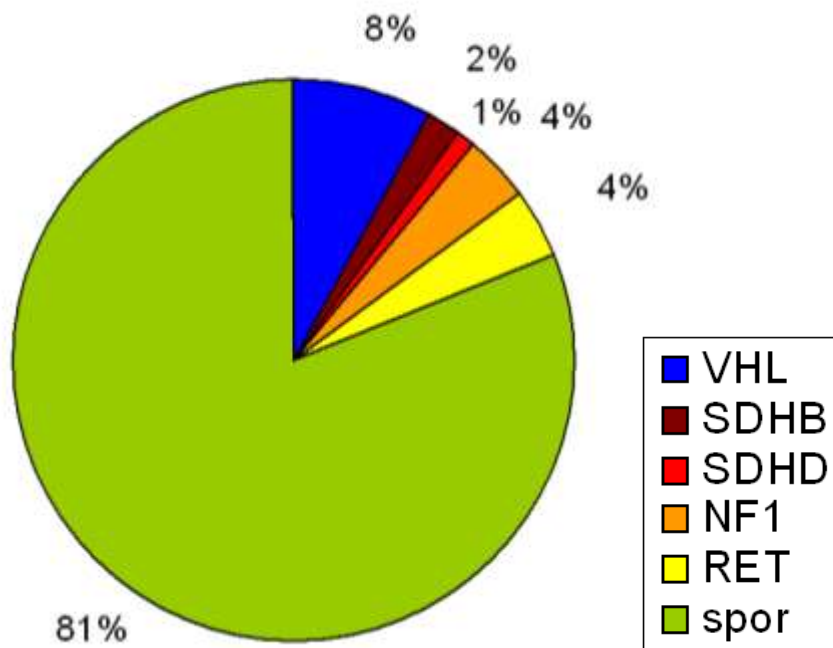




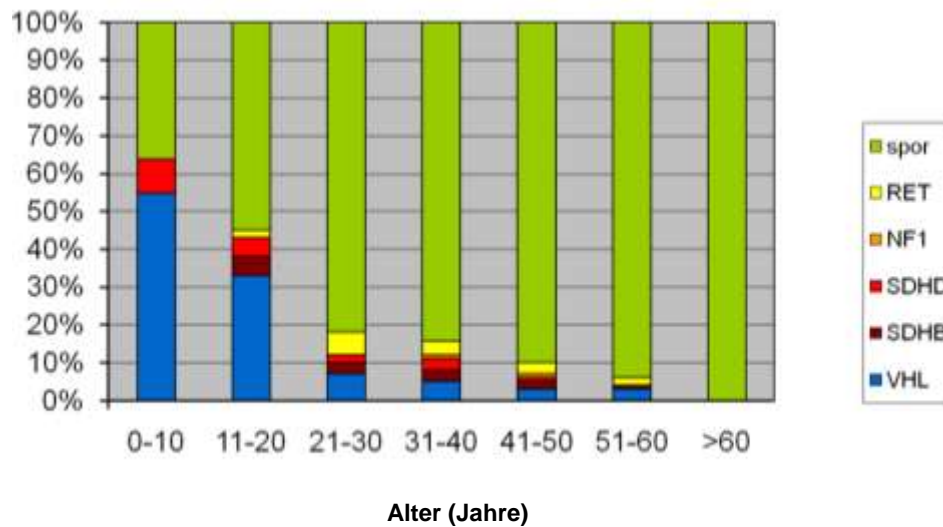
**Figure 41:** Distribution of mutations in patients with multiple glomus tumors.



**Figure 42:** Distribution of mutations in patients with malignant glomus tumors.



**Figure 43:** Distribution of mutations in patients with one-sided, benign pheochromocytoma located in the adrenal glands.



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**Figure 44:** Distribution of mutations in patients with one-sided, benign pheochromocytoma located in the adrenal glands in decades.

## 16. MULTIPLE ENDOCRINE NEOPLASIA TYPE 2 (MEN 2)

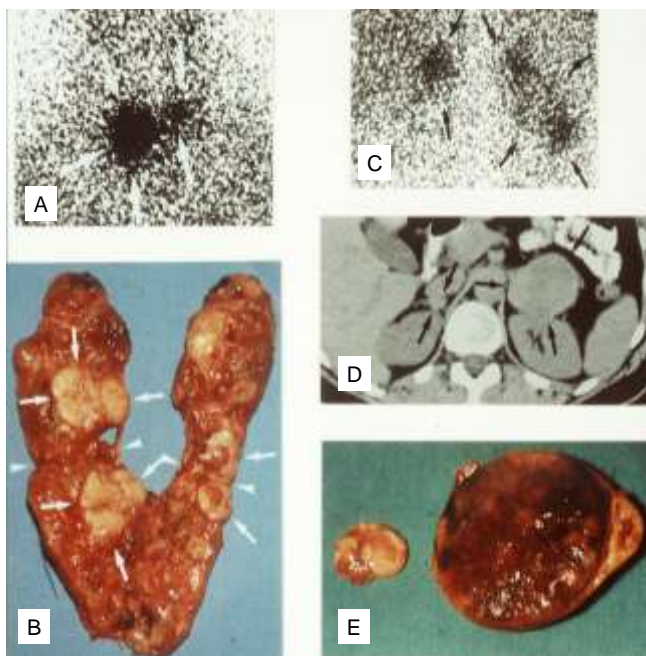
The multiple endocrine neoplasia type 2 (Figure 45) is an inherited disease that is based on mutations of the RET (rearranged in transfection) gene. Three sub-categories are being distinguished:

MEN2A: medullary thyroid carcinoma, pheochromocytoma, and hyperplasia of the parathyroid glands

MEN2B: medullary thyroid carcinoma, pheochromocytoma and constitutional anomalies with tall height as well as neuroma of the tongue, conjunctiva, and colon

FMTC: familial medullary thyroid carcinoma affecting only the thyroid gland.

Pheochromocytomas do not occur in FMTC.



**Figure 45:** Multiple endocrine neoplasia type 2 (MEN 2). 44-year old patient. A and B: Medullary thyroid carcinoma; MIBG scintigraphy (A, C) and surgery preparation (B) with double tumors (long arrows; the arrow heads point to the tissue bridge of the cut and opened preparation). C-E: bilateral pheochromocytoma (D: CT, horizontal view). From Neumann HPH. The Keio J Med 2005;5:15-21 with kind permission of the publisher (for complete reference please see section references).

Of particular interest is the prevention for medullary thyroid carcinoma (MTC). It develops from parafollicular cells of the thyroid gland, the so-called C cells that produce calcitonin. A C cell hyperplasia precedes the MTC. Medullary thyroid carcinomas metastasize into the regional lymph nodes of the neck and the thorax. More distant metastases are usually located in the bones, liver, and lungs. The treatment is difficult when distant metastases are present. The aim of preventive medicine is to detect and treat MTC early, i.e. before it forms metastases. This can be achieved with family histories and genetic analysis of family members with genetic mutations. The current recommendations for carriers of mutations predisposing for MEN2A include removal of the thyroid gland at by the age of 6. For carriers of mutations predisposing for MEN2B removal of the thyroid gland is recommended at age 1 because the MTC of MEN2B is much more aggressive. The spectrum of mutations in the RET gene is listed in Chapter 22. The majority of the mutations affect codon 634, located in exon 11. Additional mutations that predispose for MEN2A are located in codons 609, 611, 618, and 620 in exon 10. Mutations in codon 918 in exon 16 predispose for almost all cases of MEN2B.

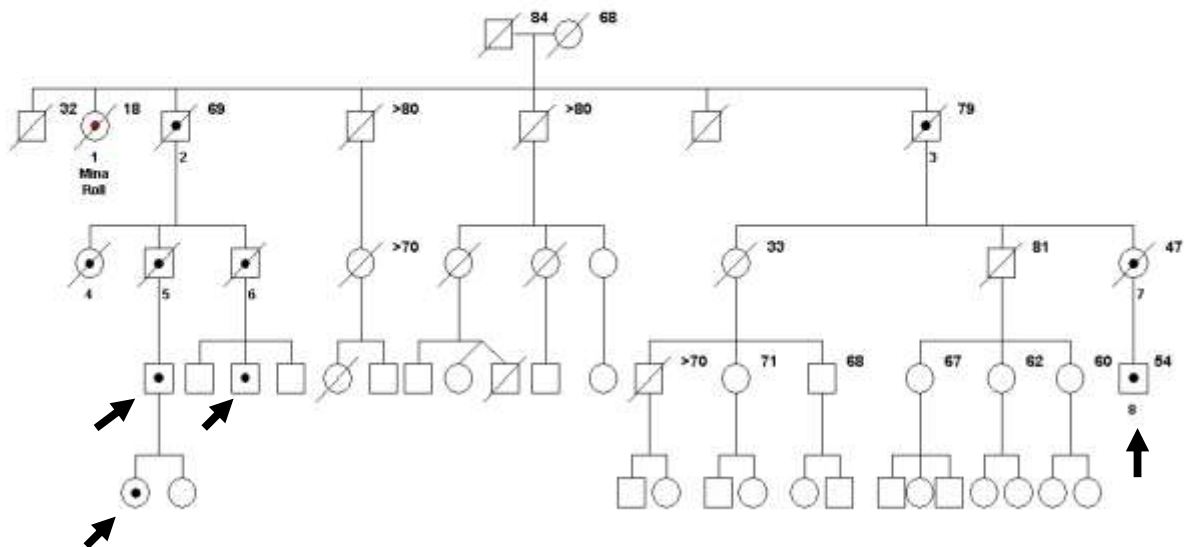
Pheochromocytomas develop in approximately 50 % of patients with MEN2A and MEN2B. Concurrent tumors in both adrenal glands as well as the development of a 2<sup>nd</sup> tumor in the contra-lateral adrenal gland years later have been described. In almost all cases with MEN2 the pheochromocytomas are located within the adrenal glands. Less common are extra-adrenal pheochromocytoma in the retroperitoneum whereas paraganglioma of the thorax and head/neck in MEN2 are very rare.

The RET mutation in the International Pheochromocytoma Registry are summarized in Chapter 22. It is advisable to perform endocrine diagnostics for MEN2 (Table 4) in patients with pheochromocytoma and mutations in the RET gene. The calcitonin levels should be determined at baseline and 2 and 5 minutes after pentagastrin stimulation (pentagastrin test). This test identifies almost all MTC. CEA is usually also elevated. To diagnose a hyperfunction of the parathyroid glands (hyperparathyroidism) Calcium and parathyroid hormone are determined. For surgery and post-operative treatment of MTC specific information should be acquired.

Table 4: Screening for multiple endocrine neoplasia type 2

- Calcitonin in the serum
- Before and 2 and 5 minutes after stimulation with pentagastrin
- Carcinoembryonic antigen (CEA) in the serum
- Parathyroid hormone, calcium, phosphate in the serum
- 24 hour Urinary metanephrens

As an example of a family history of MEN 2, the pedigree of a so-called classical family is depicted in Figure 46. Depicted is the family of the patient Minna Roll the Freiburg physician Dr. Felix Fränkel described in 1886 with bilateral tumors of the adrenal glands based on the clinical picture and histology. The mutation was confirmed in Freiburg in 2007 in living family members, which confirmed the diagnosis of bilateral pheochromocytoma in MEN2A.

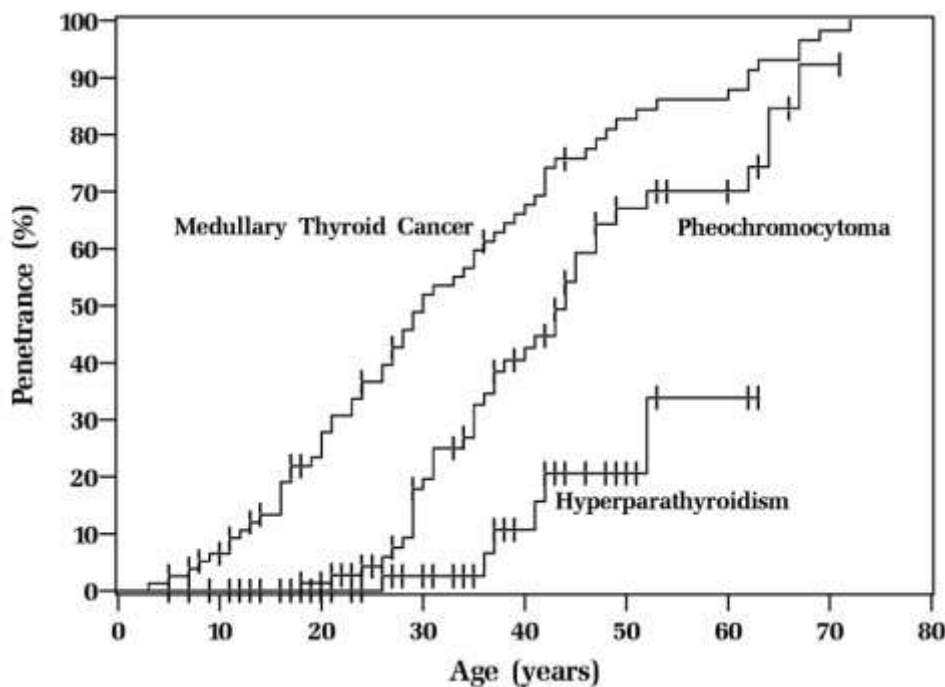


**Figure 46:** Pedigree of a “classical” family with multiple endocrine neoplasia type 2. The family history of Minna Roll was described in 1886. The mutations were confirmed in 2007 in Freiburg. Arrows indicate living family members with confirmed mutations of which Minna Roll was also a carrier: RET codon 634 Cysteine > Tryptophan (Cys634Trp or C634W). From Neumann et al. *N Engl J Med* 2007;357:1311-5, with kind permission of the publisher (for complete reference please see section references).

## Penetrance

To determine the relative risk of patients carrying mutations, a large patient population carrying this mutation and the development of the disease and single components should be analyzed. For MEN2 these components are medullary thyroid carcinoma (MTC), pheochromocytoma, and hyperfunction of the parathyroid

gland (hyperparathyroidism). For the MTC, findings of thyroid surgeries and/or the blood levels of calcitonin are collected; for pheochromocytoma, surgery findings, MRI or CT of the adrenal glands and measurements of catecholamines are collected; for the parathyroid, blood parathyroid hormone levels are collected. Risk analysis was determined for carriers of the mutation RET C634W based on analysis of 92 carriers of the mutation (Figure 47).

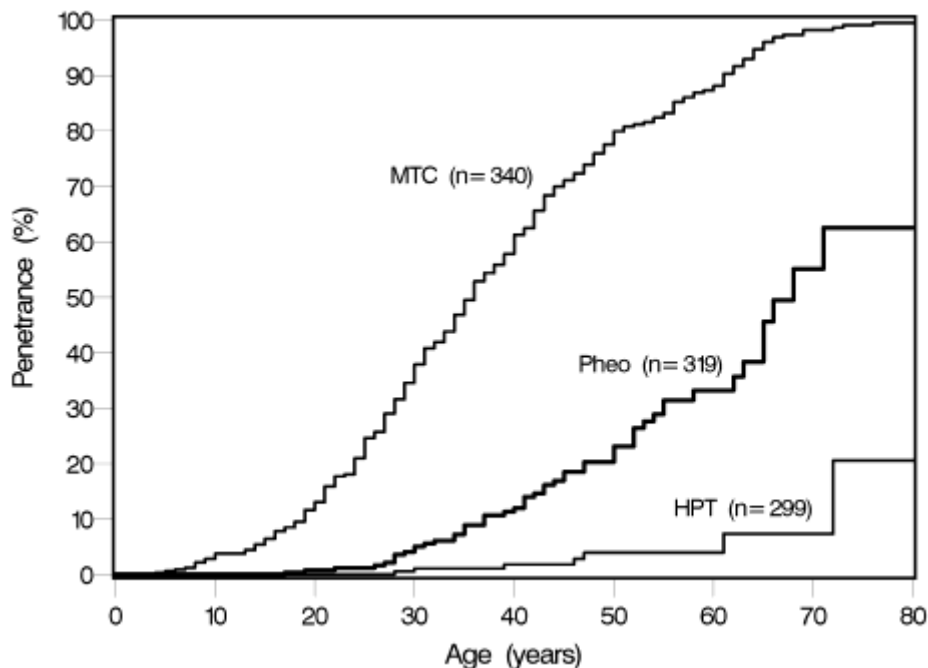


**Figure 47:** Penetrance for the presence of medullary thyroid cancer, pheochromocytoma, and hyperparathyroidism in patients with the mutation RET codon 634 Cysteine > Tryptophan (Cys634Trp or C634W). From Milos I et al. Endocrine-Related Cancer 2008 with kind permission of the publisher (for complete reference please see section references).

The penetrance for a medullary thyroid carcinoma is 52% at an age of 30 and 83% at an age of 50 years old. The penetrance for pheochromocytoma is 20% at an age

of 30 and 67% at an age of 50 years old, for hyperparathyroidism 3% at the age of 30 and 21% at the age of 50 years old.

For patients carrying mutations of the exon 10, i.e. within the codons 609, 611, 618, and 620, the penetrance has been determined by an international consortium (Figure 48). Collecting the data from 340 carriers of mutations a total of 22 different mutations were identified. There was no difference in regard to the relative risk between the different mutations. At the age of 50 the penetrance of MTC is 57%, of pheochromocytoma 23%, and of hyperparathyroidism 4 %, respectively. More detailed information on the penetrance is available in the specialized literature.#

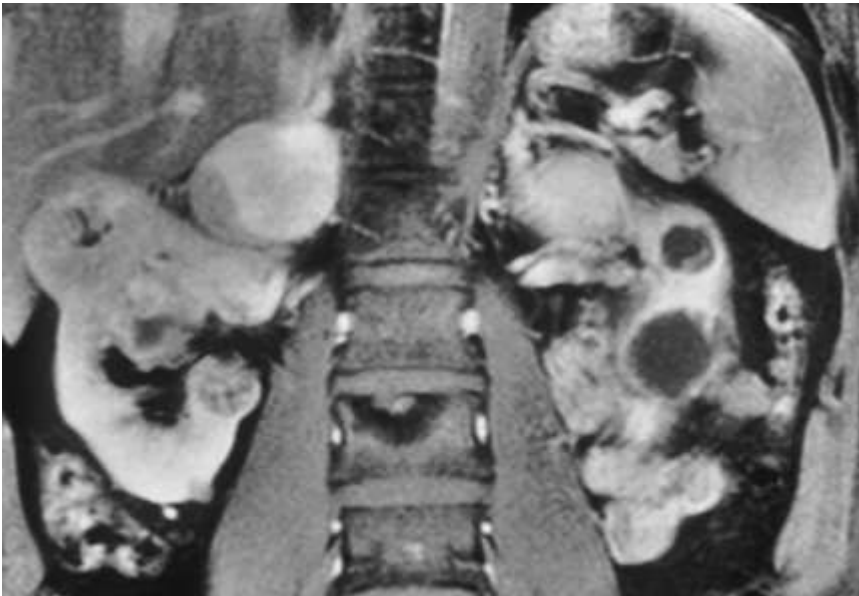


**Figure 48:** Penetrance for the presence of medullary thyroid cancer, pheochromocytoma, and hyperparathyroidism in patients with mutations of the RET gene in exon 10 (codons 609, 611, 618, 620). From Frank-Raue K et al. Hum Mutat 2011 with kind permission of the publisher (for complete reference please see section references).

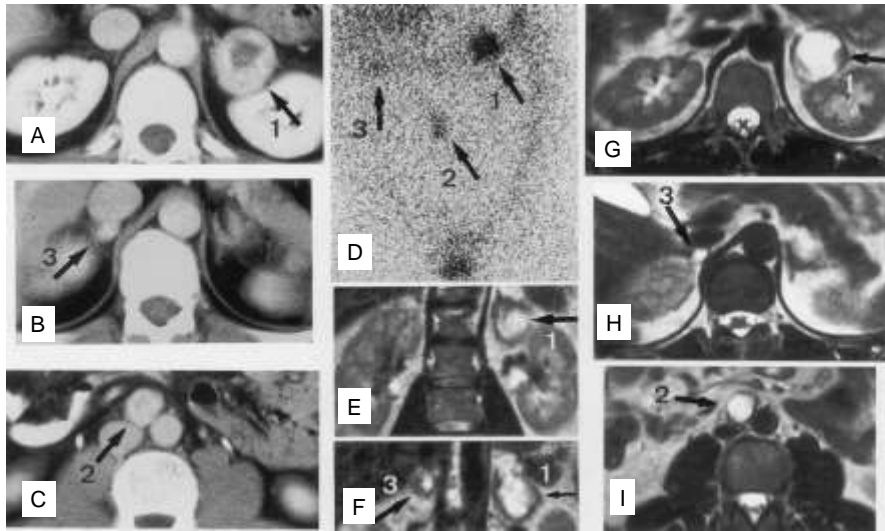


## 17. VON HIPPEL-LINDAU DISEASE

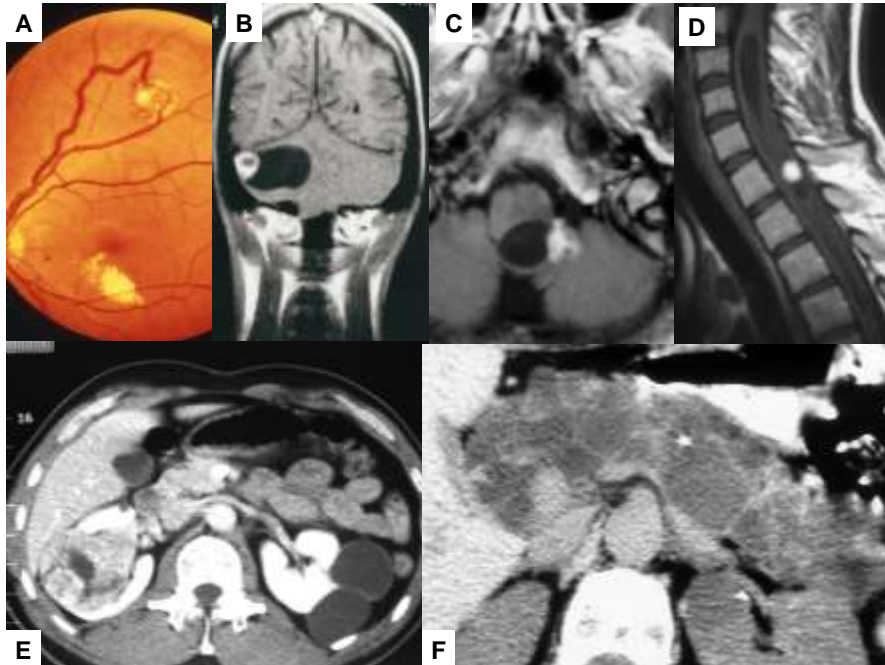
Separate advisories for patients and physicians have been published for von Hippel-Lindau disease in several languages by the VHL Family Alliance. Here, we will only describe the aspect of pheochromocytoma in von Hippel-Lindau patients. Preventive medicine offers a huge possibility in von Hippel-Lindau patients, since most of the tumors can be treated very well when diagnosed early. This particularly applies to angioma of the retina (laser therapy), hemangioblastoma of the cerebellum, brainstem, and spinal cord (neurosurgical removal), carcinoma of the kidneys (organ-sparing surgeries), and pheochromocytoma (endoscopic surgeries). The pheochromocytoma in patients with von Hippel-Lindau disease (Figures 49, 50) and the other most common tumors in this disease are shown in figure 51.



**Figure 49:** Von Hippel-Lindau disease with bilateral pheochromocytoma of the adrenal glands and bilateral partially cystic kidney carcinoma. MRI, 34-year-old patient.



**Figure 50:** 30-year-old patient with von Hippel-Lindau disease and bilateral pheochromocytoma of the adrenal glands (1, 3) and extra-adrenal pheochromocytoma in the abdomen (2). A-C: CT, D: MIBG scintigraphy (front view), E, F: coronary MRI (front view), G-I horizontal MRI. All 3 tumors were removed laparoscopically.



**Figure 51:** Changes of the von Hippel-Lindau disease outside the paraganglial system: Angioma of the retina (A), hemangioblastoma of the central nervous system: cerebellum (B, front view), brain stem (C, view from the top), spinal cord, neck region (D, lateral view), kidney carcinoma and kidney cysts (E), and multiple pancreatic cysts (F). From Neumann HP et al *Contrib Nephrol* (Karger) 2001;136:193-207 with kind permission of the publisher (for complete reference please see section references).

The von Hippel-Lindau disease is subdivided into two different types depending on the appearance of pheochromocytoma: type 1 (mostly without pheochromocytoma), and type 2 (mostly with pheochromocytoma). Further subdivision exists for type 2: mostly without kidney carcinoma (type 2A), often with kidney carcinoma (type 2B), nearly only with pheochromocytoma (type 2C).

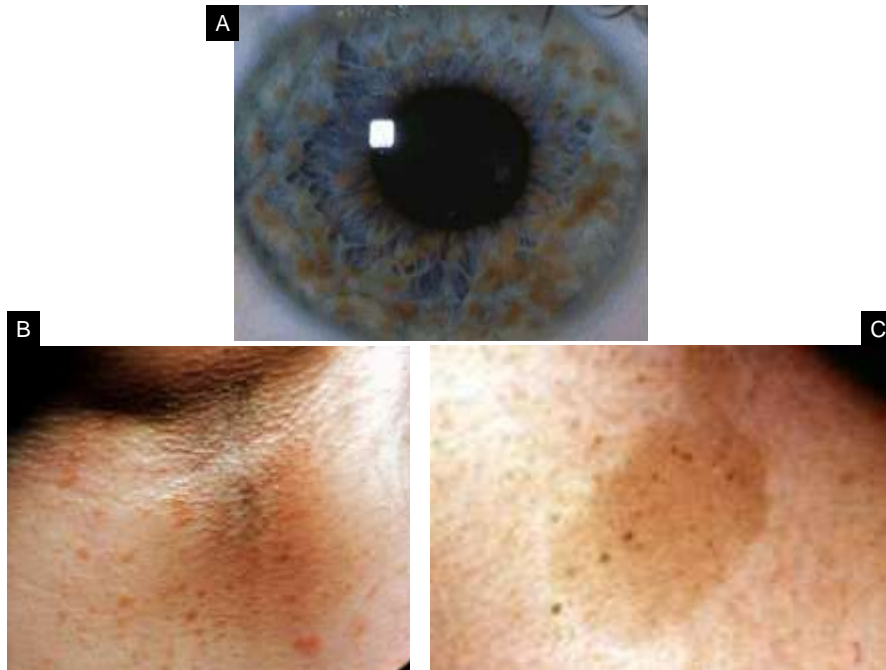
The von Hippel-Lindau disease is caused by mutations of the VHL gene. Pheochromocytomas are a result of many mutations. Mutations are detected in all exons. The mutations (identified in the Freiburg International Pheochromocytoma Registry) and the observed tumors in other organs are listed in Chapter 22. Patients with pheochromocytoma and concurrent mutations in the VHL gene should undergo the clinical tests listed in Table 5.

## 18. PHEOCHROMOCYTOMA AND NEUROFIBROMATOSIS TYPE 1 (NF 1)

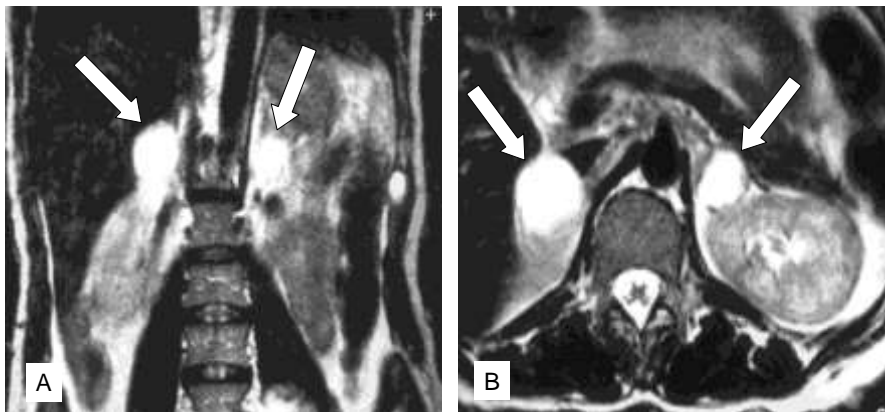
Neurofibromatosis type 1, also called von Recklinghausen's disease, is dominated by multiple neurofibromas of the skin. The disease is inherited as an autosomal dominant disorder. A high rate of spontaneous mutations occurs in the NF1 gene located on chromosome 17 (17q11.2). Patients with NF 1 develop lesions of the skin called *café au lait* spots, freckles of the axillae (armpits), or brownish nodules (Lisch nodules) of the iris (Figures 52-54). Furthermore, different benign and malignant tumors of the nervous system or endocrine organs may appear.



**Figure 52:** Neurofibromatosis with multiple neurofibroma of the skin.



**Figure 53:** Neurofibromatosis Recklinghausen. A: Lisch knot of the Iris. B: Freckle-like spots of the armpits. C: So-called Café-au-lait spots. A from Bausch et al. *J Clin Endocrinol Metab* 2006 with kind permission of the publisher (for complete reference please see section references)., B from Neumann HPH et al *The Keio J Med* 2005;5:15-21 with kind permission of the publisher (for complete reference please see section references).



**Figure 54:** Neurofibromatosis type 1 with bilateral pheochromocytoma of the adrenal glands. MRI, front view (A), horizontal view (B).

Patients with pheochromocytoma and neurofibromatosis type 1 are rare. In the Freiburg International Pheochromocytoma Registry only 5% of patients belong to this category. Likewise, in registries for neurofibromatosis type only 3% of patients have pheochromocytoma. Therefore, there are only very few reports of patients with NF1 and pheochromocytoma.

The cause of the disease is mutations in the NF1 gene. The NF1 gene consists of 57 exons and is one of the largest human genes. The analysis of this gene is very time- and cost- intensive. The large number of so-called pseudo-genes complicates the analysis. Furthermore, the analysis of large deletions is also very elaborate.

The Freiburg group published 3 articles in 2006 and 2007 investigating molecular genetic and clinical findings of patients with NF1 and pheochromocytoma. The essential and clinically important findings are: in approx. 90% of these patients a mutation within the NF1 gene can be detected. The mutation does not give any indication for a specific pattern of disease development. On the other hand there is no correlation between specific mutations of the NF1 gene and accumulation of pheochromocytoma. The third finding indicates that mutations of the NF1 gene were only identified in patients that had concurrent skin changes seen in NF1. In summary, the analysis of the NF1 gene may not be recommended for the practice due to clinical reasons and costs.

The pheochromocytomas of NF1 are usually located in the adrenal glands, in 20 % of the patients, bilateral. Twelve % of the patients develop a malignant pheochromocytoma. Only 16% of these patients had a family history for NF1.

## 19. THE PARAGANGLIOMA SYNDROME TYPE 1 TO TYPE 4

The paraganglioma syndromes (PGL) are inherited diseases that are characterized by the development of pheochromocytoma and glomus tumors. Four different types can be distinguished: type 1 was described in the year 2000, type 2 prior to 2000, and types 3 and 4 after 2000. The designation paraganglioma syndrome is based on the fact that initially only patients with glomus tumors (head and neck—paraganglioma) were described in case reports. The classification of the patients to the four types is nowadays based on molecular genetic findings. Patients with PGL1 have mutations in the SDHD gene, patients with PGL2 have mutations in the SDHAF2 gene, patients with PGL3 have mutations in the SDHC gene, and patients with PGL4 have mutations in the SDHB gene.

<b>Name</b>	<b>Gene</b>	<b>Chromosomal location</b>
Paraganglioma syndrome type 1	SDHD	11q23
Paraganglioma syndrome type 2	SDHAF2 (SDH5)	11q13
Paraganglioma syndrome type 3	SDHC	1q21-23
Paraganglioma syndrome type 4	SDHB	1q36

<b>Mutated gene</b>	<b>Disease</b>
SDHA	no name
SDHB	paraganglioma syndrome type 4
SDHC	paraganglioma syndrome type 3
SDHD	paraganglioma syndrome type 1
SDHAF2 (SDH5)	paraganglioma syndrome type 2

Table 5 Paraganglioma syndromes, current nomenclature

### *The paraganglioma syndrome type 1 (PGL1)*

Patients with paraganglioma syndrome type 1 have mutations in the SDHD gene. Mutations are either present in one of the 4 exons and can be detected by sequencing, or deletions of one or multiple exons and can be detected by quantitative multiplex PCR of short fragments (QMPSF). PGL1 is the most common paraganglioma syndrome.

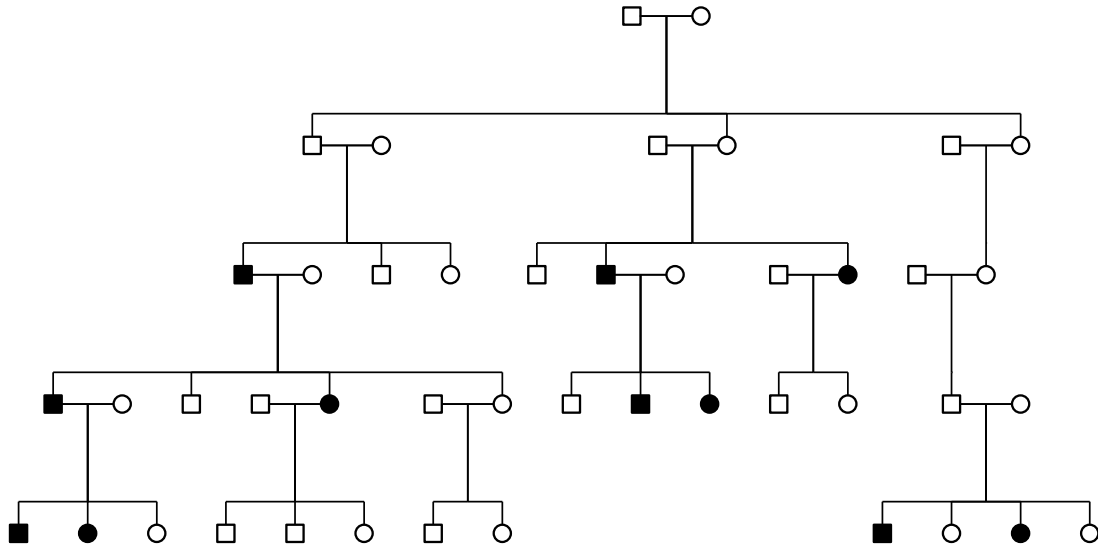
Usually, patients with PGL1 have multiple tumors, both multiple glomus tumors as well as multiple pheochromocytomas. However, mutations of the SDHD gene could also be detected in patients with a single tumor.

Over 100 persons with mutations in the SDHD gene and tumors are registered in the Freiburg Registry. The age of diagnosis ranges from 5 to 70 years of age and the average age is 30 years. Both genders are equally affected. Glomus tumors are detected in almost all patients. The majority of the patients are diagnosed with a glomus caroticum tumor. Approximately one third of the patients have multiple tumors. Approximately one fourth of the patients had pheochromocytoma, the majority of those had multiple pheochromocytomas. Approximately half of the patients with pheochromocytoma had extra-adrenal tumors located in the abdomen and one third of these patients had thoracic pheochromocytoma. Malignant pheochromocytoma or glomus tumors were only identified in 5 % of the patients.

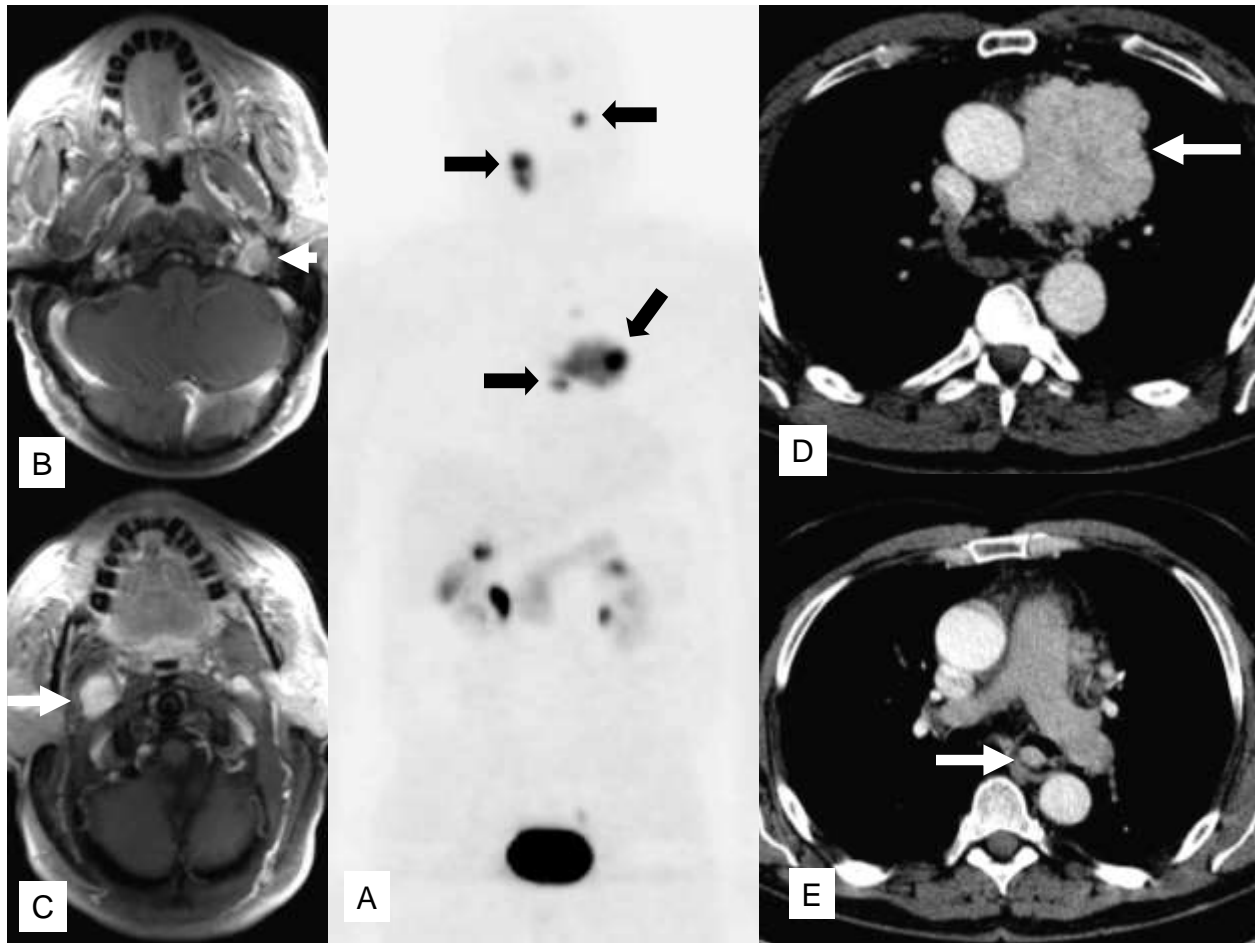
The predisposition for PGL 1 is passed on from generation to generation to theoretically 50 % of all children, i.e., it can be detected in 50 % of the children of mutation carriers. However, the disease only occurs in persons who inherited the mutations from the



father (Figure 55). This is called “parent-of-origin-effect” or sometimes (incorrectly) “maternal imprinting”. Examples for the PGL 1 are given in Figures 56 and 57.



**Figure 55:** Fictive pedigree of a family with mutations in the SDHD gene. Circles: Women, squares: Men, black: affected. Patients only developed tumors when the mutation was inherited from the father. A similar pedigree was published in Van der Mey AG et al. Lancet 1989;2:1291-1294,



**Figure 56:** 56-year-old patient with SDHD mutation. A: [ $^{18}\text{F}$ ] DOPA-PET with bilateral glomus tumors (top two arrows) and two mediastinal pheochromocytoma (two bottom arrows). B and C: Glomus tumors indicated by the top two arrows in A. D and E: Thoracic (mediastinal) pheochromocytoma indicated by the bottom arrows in A. A: Front view. B-E: Horizontal view, MRI. From Reisch N et al. *Der Internist* 2009;50:27-35 with kind permission of the publisher (for complete reference please see section references).



**Figure 57:** 36-year-old patient with SDHD mutation. Imaging after surgery for glomus tumors identified a new right-sided glomus caroticum tumor (A and C, top arrow), a left-sided pheochromocytoma (B), and a very small thoracic pheochromocytoma between pulmonary artery and aorta (D, E; D-CT, E-MRI) were detected. C: [ $^{18}\text{F}$ ] DOPA-PET shows clearly the glomus caroticum and the tumor in close proximity of the heart (arrows). In contrast, the thorax only reveals a background activity. No tumor-suspicious detection.

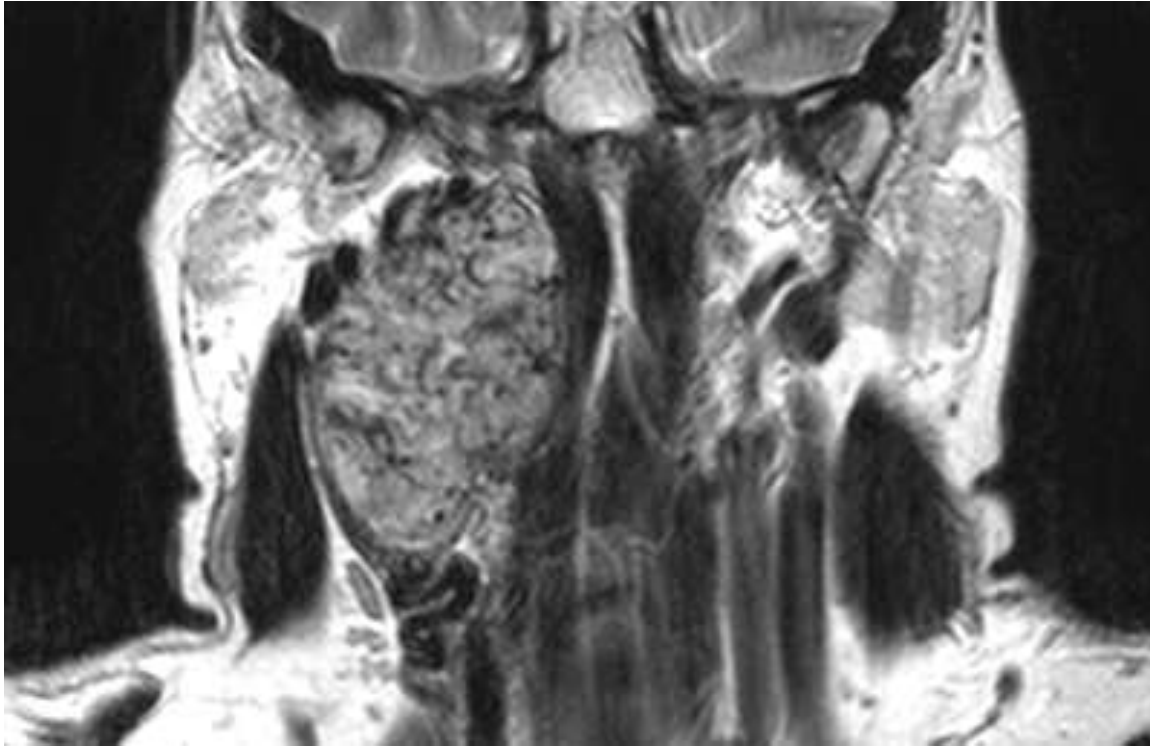
A table with the mutations of the SDHD gene detected in the Freiburg laboratory can be found in Chapter 22.

### *The paraganglioma syndrome type 2 (PGL2)*

Patients with paraganglioma syndrome type 2 have mutations in the SDHAF2 gene. Only one mutation has been described so far. The mutation is located in proximity to exon 4 and is called SDHAF2 c.232G>A (pGly78Arg). All patients with PGL2 have exclusive glomus tumors. The age at diagnosis is approximately 30 to 70 years of age and the average is approximately 40 years of age. Both genders are equally affected. PGL2 is inherited following the same parent-of-origin-effect as PGL1: disease will develop only when the mutation is transmitted from the father.

### *The paraganglioma syndrome type 3 (PGL3)*

Patients with paraganglioma syndrome type 3 (Figure 58) have mutations in the SDHC gene. Mutations are either present in one of the 6 exons which can be detected by sequencing, or deletions of one or multiple exons which can be detected by MLPA or quantitative multiplex PCR of short fragments (QMPSF). PGL3 is rare.



**Figure 58:** 37-year-old patient with SDHC mutation. Right-sided glomus jugulare tumor. Status after surgery (incomplete) and radiation therapy (ineffective). From Schiavi F et al JAMA 2005;294:2057-63 with kind permission of the publisher (for complete reference please see section references).

PGL3 is characterized by the occurrence of glomus tumors. Approximately 30 patients in the Freiburg International Pheochromocytoma-Glomus Tumor Registry have mutations in the SDHC gene. Almost all persons have a glomus tumor. Only few patients had a family history. The age at diagnosis is approximately 30 to 70 years of age and the average is approximately 40 years of age. Patients with SDHC mutations mostly cannot be distinguished from patients with glomus tumors without mutations (sporadic glomus tumors).

Analysis of a large number of patients with pheochromocytoma without confirmed mutations in the SDHC gene led initially to the conclusion that mutations in the SDHC gene don't occur in pheochromocytoma patients. However, more recent publications described mutations in the SDHC gene in these patients. These patients have either

pheochromocytoma in the adrenal glands or extra-adrenal tumors in the abdomen or thorax. Overall these cases are very rare.

PGL3 inheritance is autosomal dominant. The disease occurs in every generation and in both genders. The penetrance of the disease is presumably low which explains the lack of family history.

An example on the findings in PGL3 is given in Figure 58.

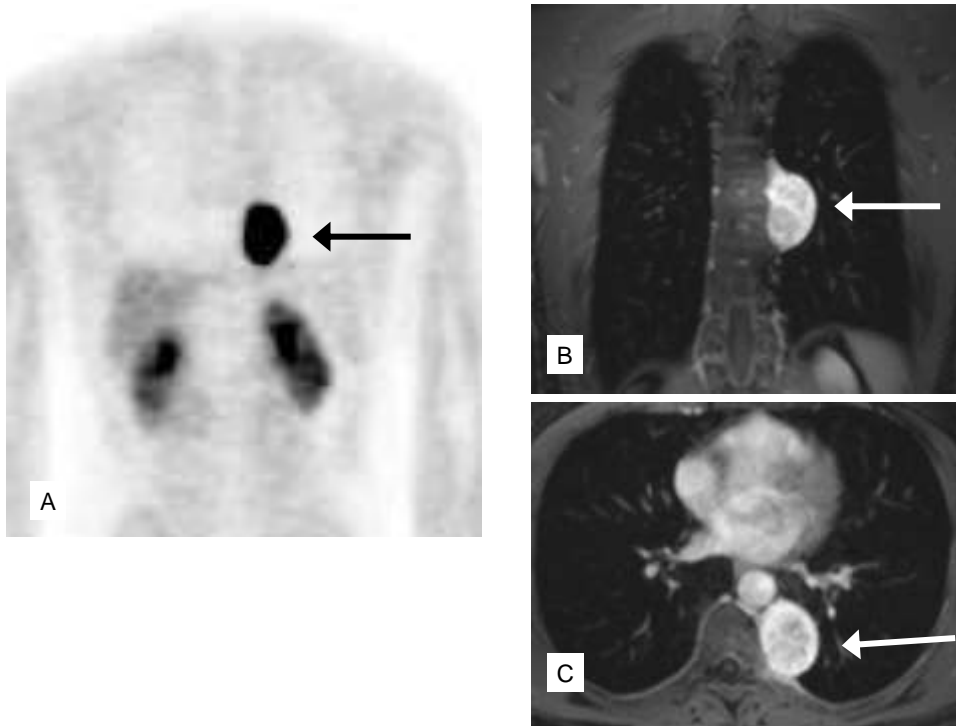
A table with the mutations of the SDHC gene detected in the Freiburg laboratory can be found in Chapter 22.

#### *The paraganglioma syndrome type 4 (PGL4)*

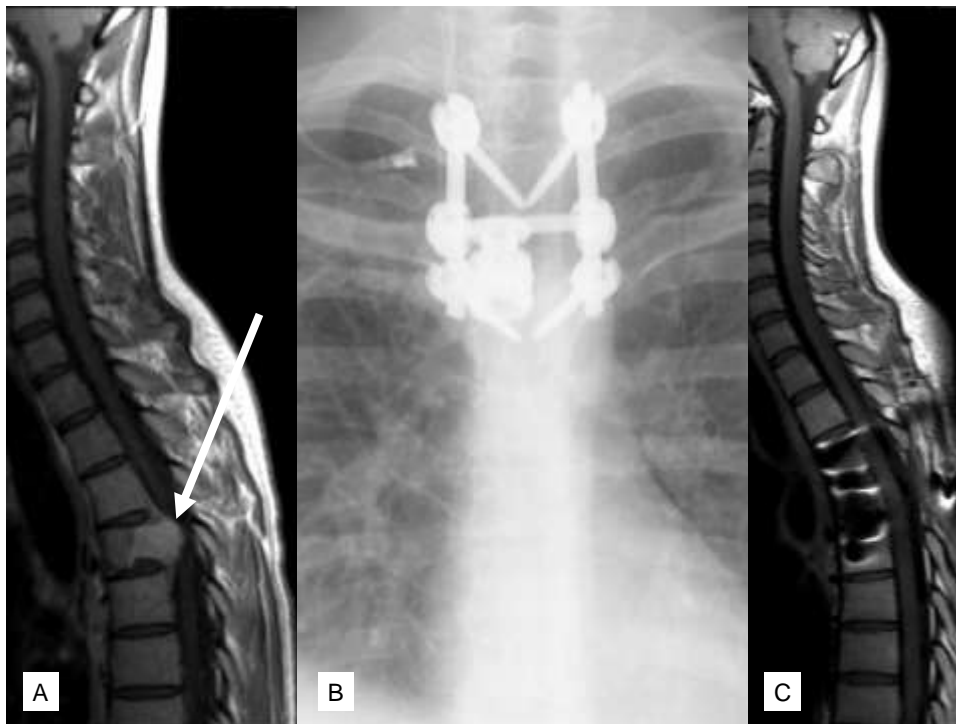
Patients with paraganglioma syndrome type 4 (Figures 59-61) have mutations in the SDHB gene. Mutations are either present in one of the 8 exons and can be detected by sequencing, or deletions of one or multiple exons and can be detected by MLPA or quantitative multiplex PCR of short fragments (QMPSF). PGL4 is the second most common paraganglioma syndrome.



**Figure 59:** 18-year-old patient with SDHB mutation with pheochromocytoma in front of the bladder. Five years of blood pressure problems, especially after urination. The tumor was detected by chance during a urological examination due to high blood pressure. The endoscopic surgery was successful and complete without opening the bladder.



**Figure 60:** 45-year-old patient with SDHB mutation with thoracic pheochromocytoma. [<sup>18</sup>F] DOPA-PET imaging (A) and MRI (B, C). The tumor is enhanced with contrast reagent. Successful endoscopic surgery.



**Figure 61:** 28-year-old patient with SDHB mutation and malignant pheochromocytoma. A: Bone metastasis (arrow) in a vertebra. The vertebra was removed and replaced by a titan artificial vertebra (B, C) without nerve damage or reduced body height.



Patients with PGL4 commonly have extra-adrenal pheochromocytoma. Frequently, patients only have one tumor.

Over 200 persons in the Freiburg International Pheochromocytoma-Glomus Tumor Registry have mutations in the SDHB gene. Only approximately two thirds of these persons developed pheochromocytoma or glomus tumor. The other one third are related mutation carriers that did not develop tumors. The age at diagnosis was 15 to 70 years of age and the average is approximately 40 years of age. Both genders are equally affected.

Glomus tumors were detected in one third of the patients. Half of these tumors were located in the glomus caroticum. Only very few patients had multiple tumors.

Pheochromocytomas are detected in half of the patients. One third of them have adrenal gland tumors. Two thirds of the patients have pheochromocytoma located in extra-adrenal areas in the abdomen. 10% of the patients with pheochromocytoma have multiple pheochromocytomas. 10% of the patients have extra-adrenal pheochromocytoma located in the thorax.

Malignant pheochromocytomas or glomus tumors were detected in almost one third of the patients.

A distinct feature of PGL4 is the occurrence of kidney carcinoma. This has been described in rare cases. During MRI of the abdomen special attention should be paid to changes to the kidneys.

PGL4 inheritance is autosomal dominant. The disease occurs in every generation and in both genders. The penetrance of the disease is presumably low which explains lack of family history.

An example on the findings in PGL4 is given in Figures 59-61.

A table with the mutations of the SDHB gene detected in the Freiburg laboratory can be found in Chapter 22.

#### *Preventive medical examinations for patients with PGL1 and PGL4*

All carriers of mutations (except children of female SDHD mutation carriers) should be subjected to a preventive medical examination. The prevention is directed towards a detection of pheochromocytoma and glomus tumors in all areas of the body, i.e. head-neck, thorax, abdomen, and pelvis area. A standard program is given in Table 6:

Table: 6 Preventive medical examinations for patients with PGL1 and PGL4

MRI of head and neck

MRI of the thorax

MRI of the abdomen including pelvis

Catecholamines or metanephrines in plasma or in 24-h-urine

This standard program may be modified based on several considerations:

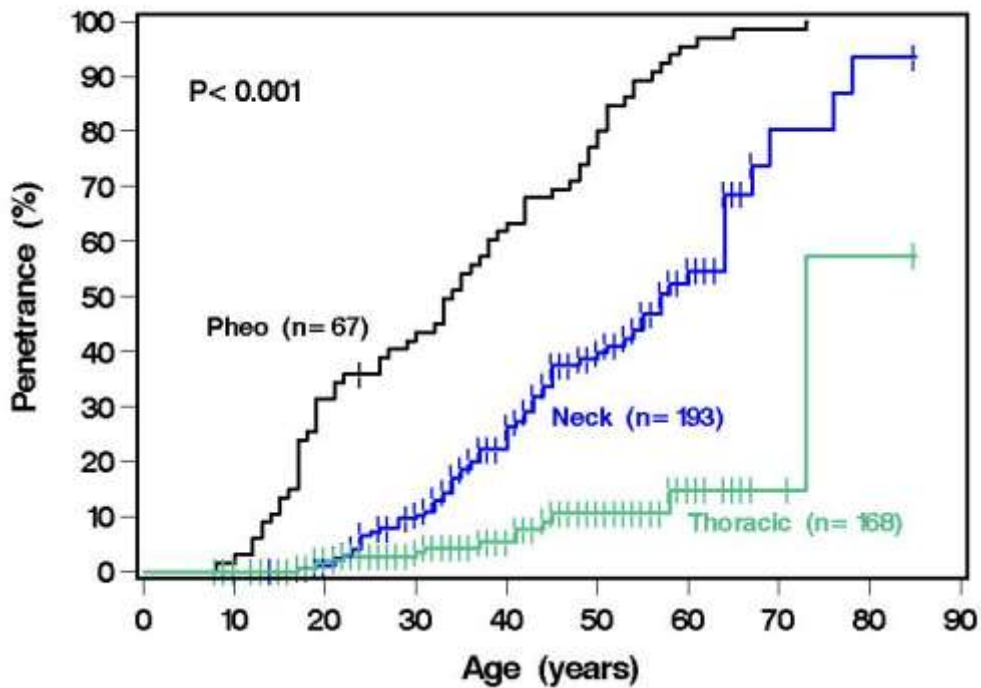
A nuclear medicine examination using [<sup>123</sup>I] MIBG, [<sup>18</sup>F]-DOPA, or Octreoscan may substitute for MRI but with a lower sensitivity.

Scintigraphy may be combined with MRI and CT such as for the so-called [<sup>18</sup>F]-DOPA PET CT.

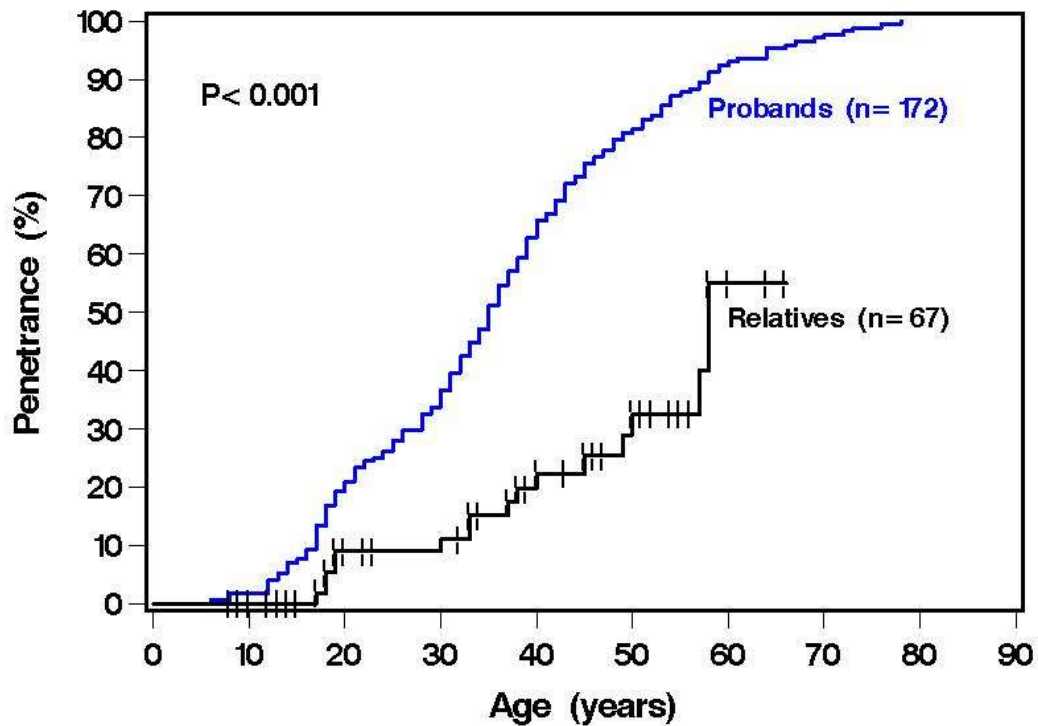
For carriers of mutations of the SDHC gene, an examination of the skull base and neck, of the thorax and of the abdomen and pelvis is recommended. Subsequently, examinations may be limited to the head-and-neck region since carriers of these mutations develop exclusively glomus tumors.

Age-related penetrance for paraganglial tumors in subjects with mutations of the genes SDHB and SDHD have been estimated for the European-American-Pheochromocytoma-Paraganglioma-Registry (Fig. 62). There was a different penetrance for tumors of the head and neck, the thorax and the abdomen (Fig. 62A). There was a similar penetrance for SDHD (Fig. 62C), but a dramatically reduced penetrance in SDHB mutation carriers (Fig. 62B).

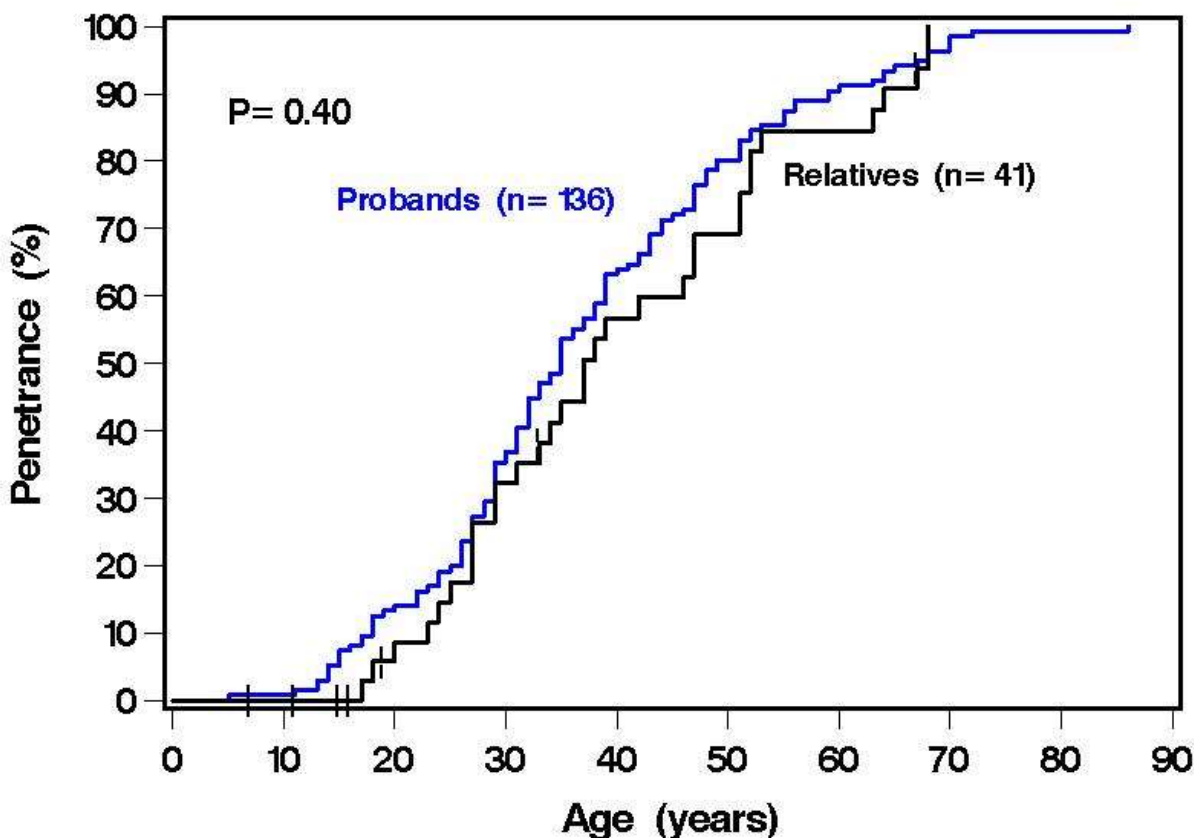
SDHB: Tumor Types (Proband and Relatives)



SDHB, All Tumors: Proband versus Relatives



## SDHD: Proband versus Relatives



**Figure 62** Age related penetrance for patients with mutations in the genes SDHB and SDHD

A: Risk calculation for adrenal pheochromocytomas, head and neck paragangliomas and abdominal extraadrenal paragangliomas in subjects with SDHB mutations. Up to age 50, approx. 75 % of the carriers developed abdominal tumors, approx. 40 % developed glomus tumors, and approx. 10 % developed tumors of the thorax.

B: Risk estimation for symptomatic patients with SDHB mutations who were the first in a given family (Probands) and their mutation-positive relatives (Relatives). Up to the age of 50 years, 80 % of the index patients developed tumors but only 30 % of the relatives.

C: Risk estimation for symptomatic patients with SDHD mutations who were the first in a given family (Probands) and their mutation-positive relatives (Relatives). There is the same risk of developing tumors for index patients and relatives.

### *Follow-up for patients with PGL1 and PGL4*

The postoperative follow-up of patients with mutations of the SDHB or SDHD genes contains the remaining standard program that wasn't done prior to surgery. It is important that patients with paraganglioma syndrome have regular follow-up. The frequencies and the extent of the follow-up differ between centers. Currently, the following recommendations are justified:

Patients with PGL1 should initially undergo an annual follow-up covering the complete program. Unless certain body regions are affected the intervals of follow-ups may be increased to 3 years in patients without manifestations.

For patients with PGL4 an extension of the intervals beyond 1 year should be carefully considered since these patients have an increased occurrence of malignant pheochromocytoma. On the other hand, many patients with PGL4 do not develop new tumors for many years. Surprisingly, it is not uncommon that relatives who carry mutations are tumor-free even at advanced age. For these persons intervals of 3 years seem sufficient.

### *Preventive and follow-up examinations for patients with PGL2 and PGL3*

Patients with PGL2 and PGL3 are rare. There are only limited experiences with preventive and follow-up examinations. This is particularly true for PGL2.

For patients with PGL3 it is recommended that after a confirmed mutation in the SDHC gene, the complete autonomous nervous system is examined using radiological or

combined nuclear medicine-radiological examinations. Multiple tumors or malignant tumors are very rare in PGL3. Therefore, follow-ups every three years seem sufficient.

Our knowledge of the paraganglioma syndromes are based on the systematic collection of data of the last 10 years. New publications could give important information and could lead to modifications of preventive and follow-up examinations.

## **20. NEW CANDIDATE GENES FOR HEREDITARY PHEOCHROMOCYTOMA.**

Patients with pheochromocytoma or glomus tumors and a family history are expected to carry a mutation in one of the described genes. Highly likely are also mutations in these genes in patients with multiple tumors or at young age at diagnosis (less than 20 years of age).

In 2009, 2010 and 2011 four new susceptibility genes have been described. These are the SDHAF2 (SDH5) gene, the SDHA gene, the TMEM127 gene and the MAX gene.

However, there are still patients in whom germline mutations could not be found in any of the meanwhile 10 susceptibility genes. Therefore, the list of susceptibility genes for pheochromocytomas, paragangliomas and glomus tumors is expected to be still incomplete.



## 21. MUTATIONS, TABLES OF MUTATIONS AND GENETIC CODE

### *Genetic background*

Molecular genetics intends to identify changes in genes which represent the hereditary predisposition and thus the cause of a disease. Specific candidate genes are analyzed for mutations. An identification of a mutation answers the question why a patient develops a tumor. The important perspective is to give good prevention to carriers of these specific mutations even before the disease becomes symptomatic. Patients should be informed, once a mutation was identified. In genetic counseling, the patients should be in detail explained about all the risks meaning the phenotype and its variations and age related penetrance. It is the challenge for the upcoming discipline of Preventive Medicine to determine the clinical screening program and the intervals of such investigations.

The following information will explain the basics of human genetics and the role of mutations.

### *Chromosomes*

The genes are located on 46 human chromosomes, 22 pairs, thus 44 autosomes, and 2 sex chromosomes. They are numbered according to their size; the largest chromosome is chromosome 1. The sex chromosomes are called X-chromosome (female) and Y-

chromosome (male). Women have 2 X-chromosomes and men one X-chromosome and one Y-chromosome as 23<sup>rd</sup> chromosome pair.

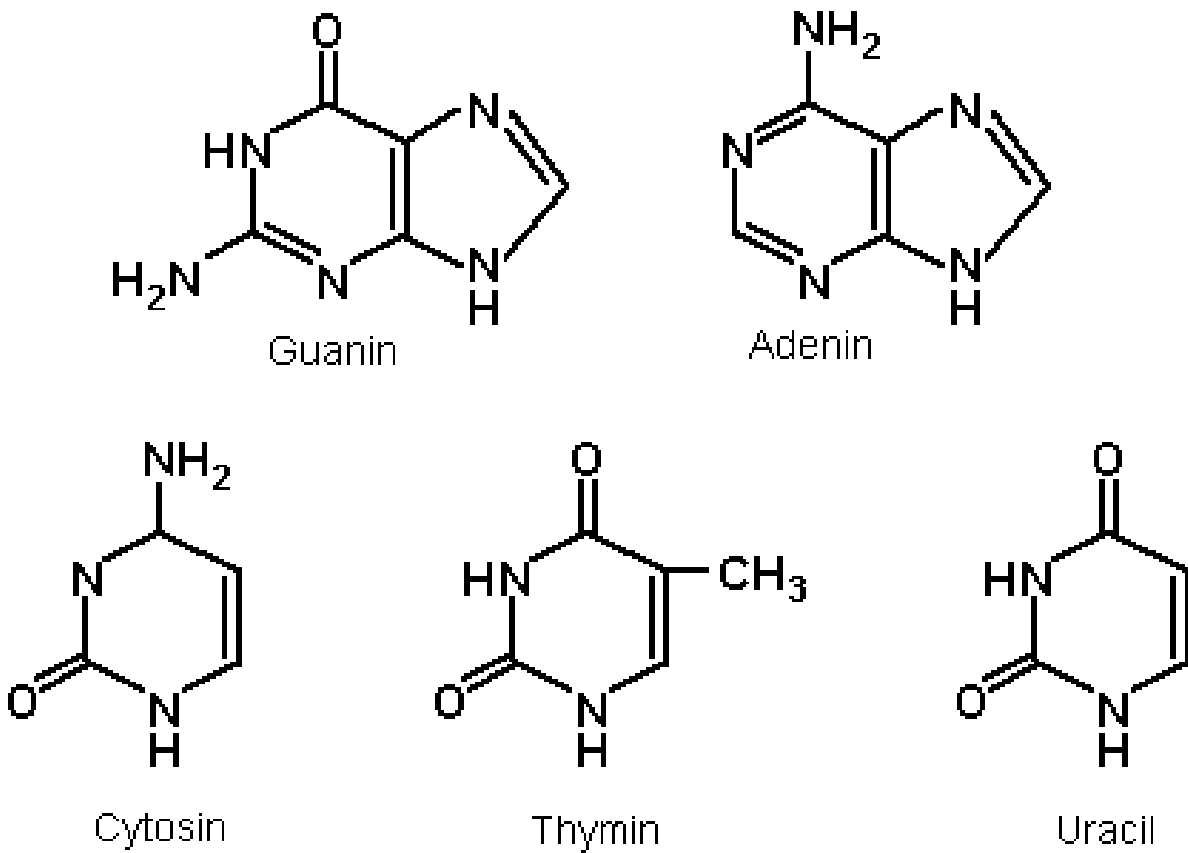
Chromosomes can be stained with certain dyes (Giemsa) that visualizes a pattern of bands. A centromere is the constricted point at which the two chromatids forming the chromosome are joined together, and that attaches it to the spindle during mitosis. Bands are numbered starting at the centromere. The chromosomes consist of centromeres, a short arm (p), and a long arm (q). Some of the bands can be subdivided into sub-bands. The bands and sub-bands are numbered beginning at the centromere. The location for the SDHD gene on #11q23 means: chromosome 11, long arm, band 23.

The chromosomes are organized structure of DNA and proteins.

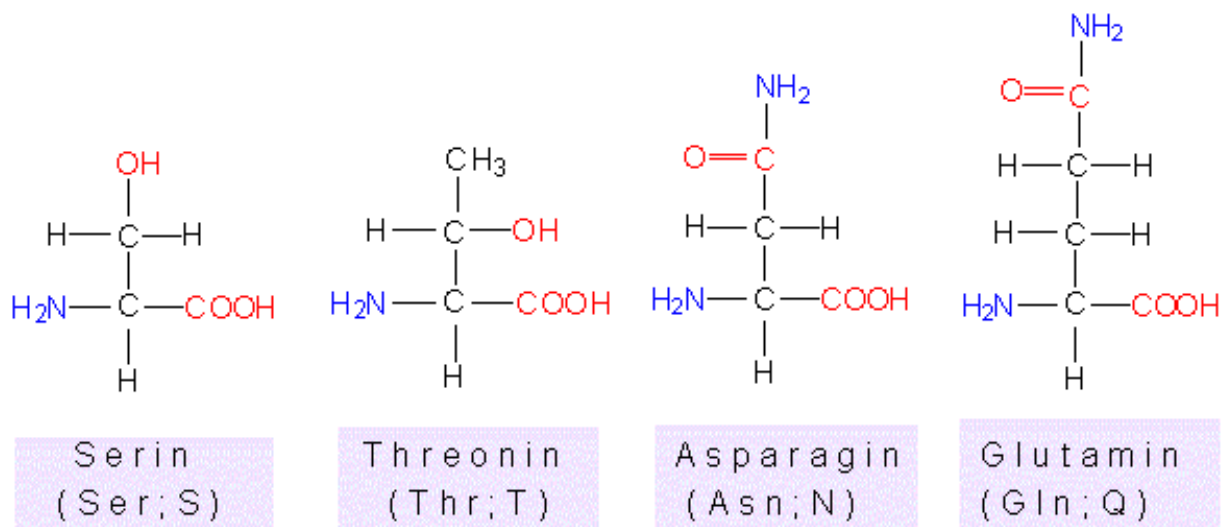
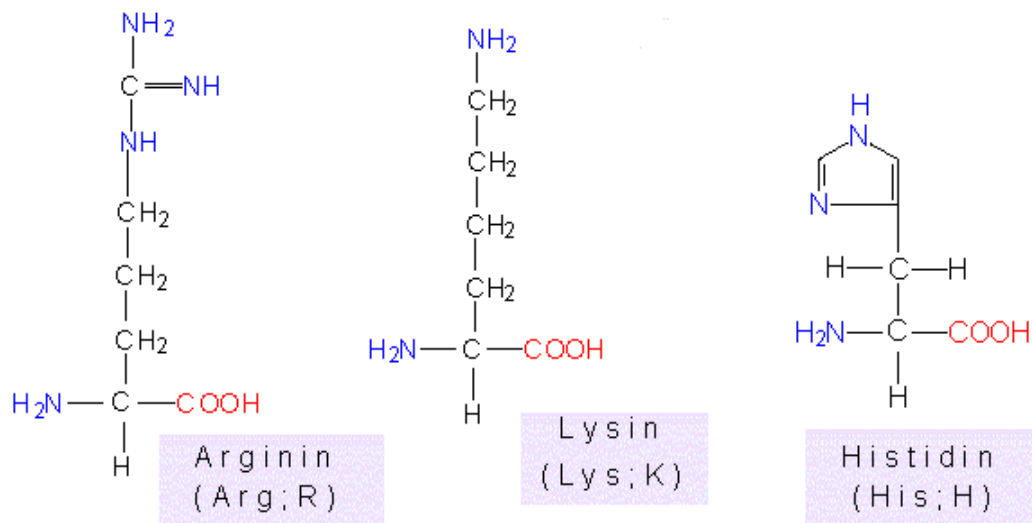
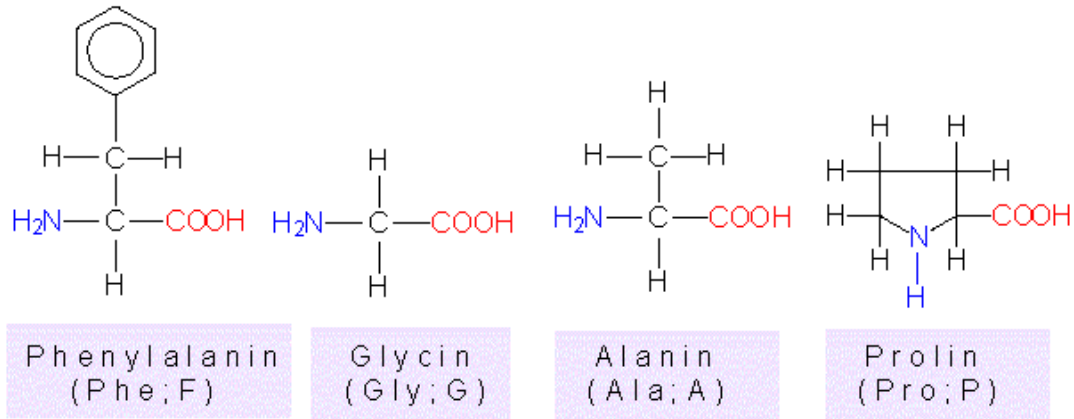
### *DNA and amino acids*

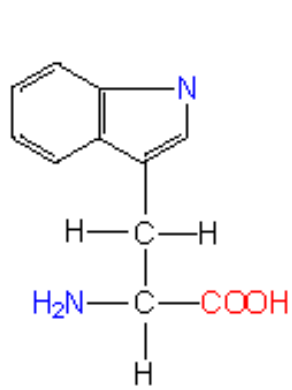
The DNA is a single piece of 2 spiral-shaped, coiled strands that are connected by phosphate and sugar residues. The DNA strand is made from alternating phosphate and sugar residues. Attached to each sugar is one of four bases: guanine (G), adenine (A), thymine (T), and cytosine (C) (Figure 64). The unit of base, sugar, and phosphate is called nucleotide. The number and the sequence of the nucleotides determine the sequence of the amino acids and ultimately the size of the protein. Twenty amino acids are encoded in the human DNA. The chemical structure of the amino acids is depicted in figure 65. Amino acids are abbreviated in a three- or one-letter code (Table 7). The

amino acids are encoded in the DNA in a way that three nucleotides encode for one amino acid. This is called the “genetic code”.

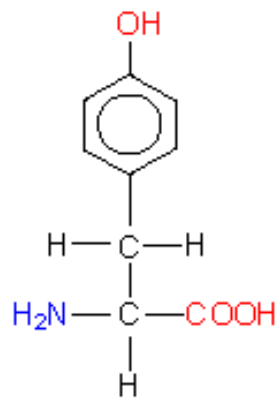


**Figure 64:** The bases of the DNA: Adenine (A), Cytosine (C), Guanine (G), and Thymine (T). Thymine is replaced by Uracil (U) in the RNA.

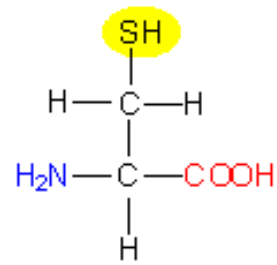




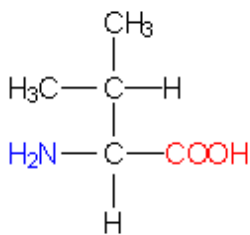
Tryptophan  
(Trp; W)



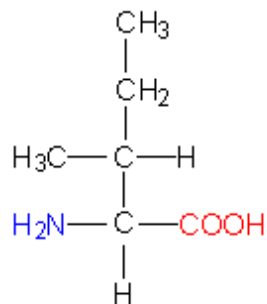
Tyrosin  
(Tyr; Y)



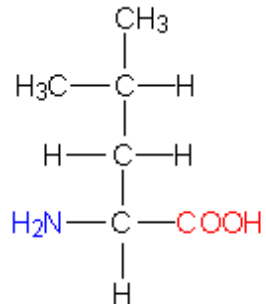
Cystein  
(Cys; C)



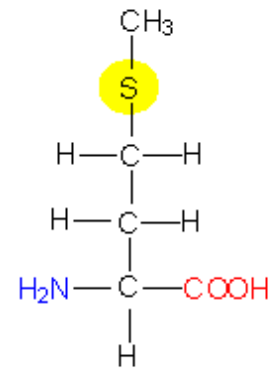
Valin  
(Val; V)



Isoleucin  
(Ile; I)



Leucin  
(Leu; L)



Methionin  
(Met; M)

**Figure 65:** The chemical structure of the essential amino acids.

Table 7: Abbreviations of the amino acids

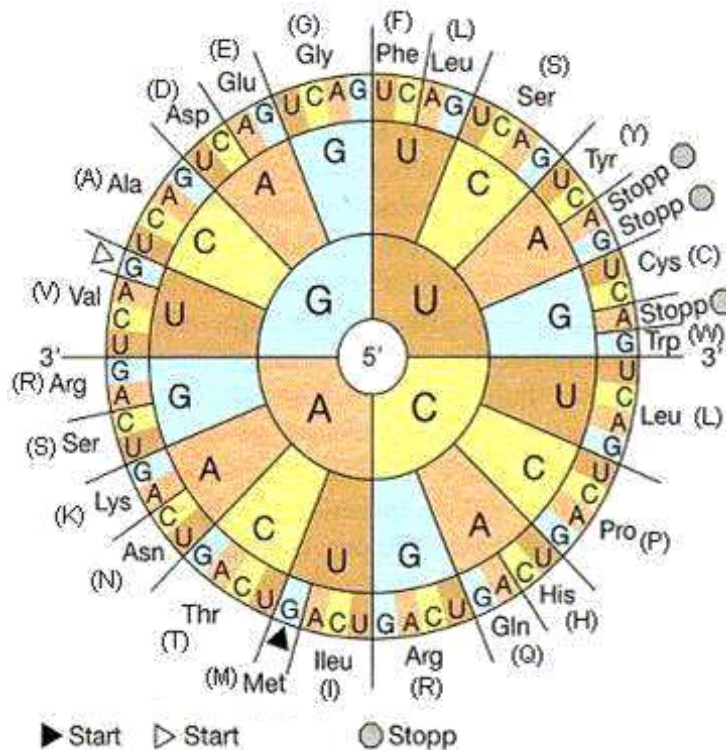
Amino Acid	3-letter code	1-letter code
Alanine	Ala	A
Arginine	Arg	R
Aspartic acid	Asp	D
Asparagine	Asn	N
Cysteine	Cys	C
Glutamine	Glu	E
Glutamic acid	Gln	Q
Glycine	Gly	G
Isoleucine	Ile	I
Histidine	His	H
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

### *The genetic code*

The genetic code is the basis of modern human genetics and for many important biological and medical questions. Changes in the genetic code lead to the generation of abnormal proteins. Smallest changes may have important consequences.

The genetic code is defined by the sequence of the bases of the DNA. Three bases, for example, ATC, TCC, or GGG define (also called “encode”) one amino acid (AA). The bases A, T, C, and G can theoretically form 64 different combinations of three bases, the so-called triplets. Therefore, there are many more triplets than the 20 amino acids.

The genetic code also contains the information for the start and end of the proteins. The start is the amino acid methionine, i.e. the code ATG. The end is encoded in the so-called stop codons: TGA (also called “opal”, TAA (“ochre”), and TAG (“amber”). Therefore, there are 60 triplets available for the remaining 19 amino acids. Thus, some amino acids can be encoded by different triplets. The fact that some amino acids are encoded by several triplets is called the degeneration of the genetic code (Figure 66).



**Figure 66:** The genetic code. The bases of the RNA are inscribed into the colored areas. The triplets can be read from the center to the periphery. The triplet CAC for instance encodes for the amino acid histidine (three-letter code: His, one-letter code: H). The amino acids are inscribed into the outer circle with their three- and one-letter codes. Since uracil (U) is present in the RNA instead of thymine (T) in the DNA, all U should be translated into T in the schemata. Please also refer to table 7 for the amino acid abbreviations. From: *Klassische und molekulare Genetik - Ein Lehrbuch* von Bresch C., Hausmann R. - Berlin / Heidelberg / New York (Springer) 1970 with kind permission of the publisher (for complete reference please see section references).

## *DNA, RNA, exons, introns, promoter*

The genomic DNA is the DNA that is present in the cell nucleus of eukaryotes, as well as small amounts in mitochondria. Thus, the white blood cells, also called leukocytes, have a nucleus and therefore contain genomic DNA. Genomic DNA is required for genetic testing. Thus, genetic testing can be performed using blood samples.

The information for the assembly of the protein has to be transported from the nucleus to other structures within the cells. In order to transport the information out of the nucleus, genomic DNA is translated into RNA (ribonucleic acid) in a complementary fashion. RNA transports the information out of the nucleus and is therefore also called “messenger” RNA (mRNA). The RNA is used to assemble the proteins in the cytosol. The RNA contains uracil (U) instead of thymine (T).

Genes consist of several, larger DNA segments with certain structural features. These segments are called promoter, exons, and introns. Most genes consist of several exons and therefore several introns which are numbered. The promoter is responsible for turning on and turning off a gene. The first exon mainly starts with the start codon (ATG=methionine). The last exon ends with a stop codon (TGA, TAA, or TAG). Only the exons contain the information for the assembly of a certain protein. The importance of the introns is largely unknown. The mRNA is the “translation” of the DNA of all the exons of a given gene. The information of all the exons has to be joined. This process is called “splicing”. A splice site is located at the beginning and end of each intron. These splice sites consist of two nucleotides (2 bases plus sugar and phosphate residue): cytosine and guanine (CG) at the beginning of each intron and adenine and guanine



(AG) at the end of each intron. If one would translate the mRNA back into the DNA, it results in a DNA only containing the coding information which is also called cDNA (complimentary DNA). The cDNA of all known genes can be looked up in special databases on the internet.

#### *DNA variations and their detection within the cDNA and the codons*

The order of the bases is called sequence and the analysis of the order and correct identification of the bases is called sequencing. Sequencing is used to identify a normal sequence or changes in the sequence (so-called "variations"). The normal sequence is also called "wild type". If a variation is identified this variation ought to be localized. The counting of the bases of the cDNA is used. The nomenclature contains the gene name, "c." for bases of the cDNA, the number, the normal base, the symbol ">" for substitute, and the detected base. VHL c. 505 T>C, for example, means that in the VHL gene the base thymine at position 505 of the cDNA is substituted with cytosine. In case the variation affects the splice site, the number of the last or first base of the exon is used and +1, +2, or -2, -1 is used, respectively. VHL c. 676+2 T>G for example means that in the VHL gene the 2<sup>nd</sup> base of the splice site following the base 676 of the cDNA is changed from thymine to guanine.

The changes of the bases ought to be analyzed for their location and importance within the codons. The numbering of the codons follows the amino acids in the cDNA. The nomenclature contains "p." for protein followed by the one-letter or three-letter

abbreviation for the normal amino acid, the number of the amino acid, and the new amino acid. VHL p. A103L for example means that in the VHL protein the amino acid alanine at position 103 is substituted with leucine. VHL p. Ala103Leu has the identical meaning. Changes of one base within a codon may lead to different outcomes: 1. Amino acid change: TGC>TCC (cysteine to serine; p.Cys55Ser). 2. Stop codon: TGC>TGA (cysteine to opal=stop or X; p.Cys55X). 3. No amino acid change: TGC>TGT (cysteine to cysteine; p.Cys55Cys).

### *Mutations and Polymorphisms*

The term “mutation” is not used consistently. In this brochure and in the general usage “mutation” is used for a change of the gene that leads to a disease. The neutral term “variation” distinguishes mutations and polymorphisms (DNA changes that do not cause diseases). The spectrum of mutations is large. Mutations may affect the substitution of a single base, which is also called point mutation, or deletions of large regions, or complex rearrangements.

### *DNA changes (variations) that are mostly considered mutations*

DNA changes that are mostly considered to be pathogenic, meaning to be mutations, are stop codons, small within an exon located deletions or small insertions. Large

deletions comprising one entire or even more exons and rearrangements of the gene are also pathogenic.

Most mutations are point mutations that either lead to amino acid changes or to a stop codon. There is no general agreement regarding the criteria when a missense DNA variant is pathogenic. There are a number of prediction programs, so-called *in silico* analyses which support the interpretation pro or against pathogenic. In addition there are DNA sequences within the genes which are highly conserved among different species. If a missense variant affects the highly conserved DNA sequences, it is very likely to be pathogenic. Other arguments are cosegregation of the disease with DNA missense variants and checking blood DNA of healthy controls for a given DNA variant. Of these aids as many as possible should be used.

This brochure summarized mutations as follows: mutations that lead to truncations are called “truncating mutations” and mutations without truncations are called “non-truncating mutations”.

### Truncating mutations

1. Stop codon mutations: these mutations affect a base and change a triplet into one of the following triplets: TAA (ochre), TAG (amber), or TGA (opal). Ochre, amber, or opals are changed to an “X”, e.g. Cys13X, in this case the protein is truncated after amino acid 12.

2. Splice site mutations: usually one nucleotide is changed either at position one or two following an exon or in front of the next exon, e.g. gene x c.553+2T>G. As a consequence, the exon composition will be changed in this protein.
3. Frame-shift mutations: Insertion or deletion of one, or two nucleotides (or 4, 5, 7, 8, 10, 11, ect. nucleotides) changes the frame of translation of the protein. Insertion of an A at position 5 changes ATG-TTG-CCG-TGC-CCT-AAG to ATG-TAT-GCC-GTG-CCC-TAA-G. The 6<sup>th</sup> codon changes therefore to TAA which is a stop codon. The mutation on the protein level is assigned p.Leu2Tyrfs6X: the amino acid leucine on position 2 changes to tyrosine by a frame-shift (*fs*) and the 4<sup>th</sup> codon changes therefore into a Stop codon (X). Some insertions or deletions do not lead to a Stop codon but to a change of the splice site which also leads to a different protein.
4. Large deletions and rearrangements also lead to a shortening of the protein. The confirmation of the missing exon is performed using MLPA or QMPSF. The exact breakage and restructuring are not defined in detail. The analysis of the VHL gene by the Freiburg laboratory determined that large deletions are variable from family to family.
5. Mutations with insertions or deletions of one or more codons are rare. It is not completely clear if these mutations may lead to the development of the disease but it is assumed as such.

#### Non-truncating mutations (missense mutations)

Missense mutations are changes in amino acids that lead to the development of the disease. Usually, one nucleotide is substituted with another nucleotide (point mutations). Sometimes two or three bases are substituted. A good example is the mutation of codon 918 of the RET gene, RET p.C634W or VHL p.Y98H. It applies to both mutations that only carrier of these mutations in affected families develop the disease. This is called co-segregation. Furthermore, the mutations could not be detected in normal blood donors. Both requirements should be fulfilled before missense DNA variants are assigned as mutations.

## **22. CRITERIA OF QUALITY FOR CENTERS FOR PHEOCHROMOCYTOMA AND GLOMUS TUMORS**

Patients with pheochromocytoma and paraganglioma should be treated in medical centers with special experience in this area. It is necessary but not sufficient that the knowledge presented in this brochure exists in such a center. Considerable practical experience is also necessary. Since this disease is rare, the number of newly diagnosed patients per year is not very high. A minimum of 10 patients with pheochromocytoma per year should be diagnosed. Even some large medical centers will not reach this number which is concerning for the patients. Taking into consideration that different physicians diagnose and operate on the patients, it is understandable that some patients have unsatisfying experience. The integrative preventive medical examinations should include molecular diagnostic and consultations. These modern methods of analysis require specialized laboratories, genetic consultation, and clinical support as part of preventive medicine. Patients will certainly welcome that specialized centers treat them according to these guidelines and will even willingly accept long commutes to these centers. Therefore, the adequate treatment of patients with pheochromocytoma in integrated interdisciplinary medical centers is recommended and should be the standard of care for the future.

## **23. TABLES OF MUTATIONS DETECTED IN THE FREIBURG LABORATORY**

In the following tables, mutations of the genes RET, NF1, VHL, SDHB, SDHC, and SDHD are listed. Mutations were identified by the Freiburg laboratory and are associated with Pheochromocytoma or glomus tumors.

Mutation	Amino acid	Exon	Localization
NF1 c. 61-1 G>A	Splice defect	2	Cutaneous Neurofibroma
NF1 c. 269 T>C	L90P	3	Cutaneous Neurofibroma
NF1 c. 277 T>C	C93R	3	Cutaneous Neurofibroma
NF1 c. 1062+2 T>C	Splice defect	7	Cutaneous Neurofibroma
NF1 c. 1466 A>G	Y489C	10b	Cutaneous Neurofibroma
NF1 c. 1580 del C	T527LfsX29	10c	Cutaneous Neurofibroma
NF1 c. 2023 ins G	T676NfsX24	13	Cutaneous Neurofibroma
NF1 c. 2409+1 G>C	Splice defect	15	Cutaneous Neurofibroma
NF1 c. 2849 ins TT	Q950HfsX5	16	Cutaneous Neurofibroma
NF1 c. 3826 C>T	R1276X	22	Cutaneous Neurofibroma
NF1 c. 4077 del T	Q1360NfsX25	23-2	Cutaneous Neurofibroma
NF1 c. 5537+1 G>T	Splice defect	29	Cutaneous Neurofibroma
NF1 c. 6641+1 G>A	Splice defect	35	Cutaneous Neurofibroma
NF1 c. 6795 ins C	S2266QfsX20	37	Cutaneous Neurofibroma
NF1 c. 6858+2 T>C	Splice defect	37	Cutaneous Neurofibroma
NF1 c. 7337 C>G	S2446X	41	Cutaneous Neurofibroma
NF1 c. 7739 C>G	S2580A	44	Cutaneous Neurofibroma
NF1 c. 7833 T/A	D2611E	45	Cutaneous Neurofibroma

Table 8: Selected mutations of the NF1 gene that were identified in the Freiburg laboratory.

The mutation NF1c.2849 ins TT was homozygous.



<b>Mutation/Codon</b>	<b>Amino acid</b>	<b>Exon</b>	<b>Associated lesions/disease</b>
RET 609 5 several mutations	C609R or G or S or F	10	Medullary thyroid carcinoma HPT only for C609S
RET 611 3 several mutations	C611Y or W or F	10	Medullary thyroid carcinoma HPT only for C611Y
RET 618 6 several mutations	C618S or R or G or Y or F	10	Medullary thyroid carcinoma HPT only for C618T
RET 620 4 several mutations	C620R or G or S or F	10	Medullary thyroid carcinoma HPT only for C620R
RET 634 TGC>CGC	C634R	11	Medullary thyroid carcinoma
RET 634 TGC>TAC	C634Y	11	Medullary thyroid carcinoma
RET 634 TGC>TCC	C634S	11	Medullary thyroid carcinoma
RET 634 TGC>TGG	C634W	11	Medullary thyroid carcinoma
RET 634 TGC>TTC	C634F	11	Medullary thyroid carcinom
RET 790 TTG>TTT	L790F	13	Medullary thyroid carcinoma
RET 918 ATG>ACG	M918T	16	Medullary thyroid carcinoma of Marfan-like nature, Mucosal Neuroma

Table 9: Mutations in patients with Multiple Endocrine Neoplasia Type 2 and Pheochromocytoma. Further information to mutations in exon 10 can be found in Frank Raue K et al. Hum Mutat 2010;32:51-8.

Mutation according to old numbering	Mutation according to new numbering	Amino Acid	Exon	Published on the Internet	Pheo-patients/ total number of mutations carrier in Freiburg	Associated lesions for the given mutations
VHL 404 G>C	191 G>C	R64P	1	*	2/4	none
VHL 406 T>A	193 T>A	S65T	1	-	1/1	none
VHL 406 T>C	193 T>C	S65P	1	*	1/1	E, C, K, P
VHL 407 C>A	194 C>A	S65X	1	*	1/3	E, C, K, P
VHL 407 C>T	194 C>T	S65L	1	*	1/5	E, C, K, P
VHL 416 C>G	203 C>G	S68W	1	*	1/3	none
VHL 421 G>T	208 G>T	E70X		*	1/3	C, K, P, I
VHL 430 C>T	217 C>T	Q73X	1	*	1/3	E, C, K, P
VHL 437_439 del TCT	224_226 del TCT	76delF	1	*	1/14	E, C, K, P, I
VHL 442 T>G	229 T>G	C77R	1	-	1/1	none
VHL 446 A>G	233 A>G	N78S	1	*	1/3	E, C, K, P
VHL 449_454 del GCAGTC	236_241 del GCAGTC	R79S80del	1	-	1/2	E, C, P
VHL 452 G>A	239 G>A	S80N	1	*	1/2	E, C, P
VHL 452 G>T	239 G>T	S80I	1	*	1/3	E, C
VHL 453 T>G	240 T>G	S80R	1	*	1/7	E, C, K, P, I
VHL 457 C>G	244 C>G	R82G	1	-	1/1	K
VHL 463 G>A	250 G>A	V84 M	1	-	1/1	none
VHL 469 C>G	256 C>G	P86A	1	*	2/2	E
VHL 469 C>T	256 C>T	P86S		*	1/3	E, C, K, P
VHL 479 T>C	266 T>C	L89P	1	*	1/10	E, C, K, P, I

VHL 490 G>A	277 G>A	G93S	1	*	4/4	none
VHL 490 G>C	277 G>C	G93R		-	2/2	E
VHL 490 G>T	277 G>T	G93C	1	-	3/6	E, C, K, P
VHL 493 G>T	280 G>T	E94X	1	*	1/4	E, C, K
VHL 500 ins A	287 ins A	P97AfsX35	1	-	1/1	E, C, P
VHL 505 T>C*	292 T>C	Y98H	1	*	81/208	E, C, K, I
VHL 532 C>A	319 C>A	R107S	1	-	2/2	E, C
VHL 532 C>G	319 C>G	R107G	1	-	1/2	None
VHL 553 G>A	340 G>A	G114S	1	*	5/8	E, C, I
VHL 553+1 G>T	340+1 G>T	Splice Defect	1	*	3/5	E, C, K, P
VHL 557 A>G	344 A>G	H115R	2	*	1/5	E, C, K, P
VHL 560 T>C	347 T>C	L116P	2	-	1/2	none
VHL 566 T>G	353 T>G	L118R	2	*	1/1	E
VHL 570 C>G	357 C>G	F119L	2	*	3/5	E, C, I
VHL 575 A>G	362 A>G	D121G	2	*	1/4	E, I
VHL 577+578 GC>AT	364+365 GC>AT	A122I	2	-	1/1	E, I
VHL 584 C>T	371 C>T	T124I	2	-	3/5	E, I
VHL 589 G>A	376 G>A	D126N	2	-	1/3	none
VHL 601 G>T	388 G>T	V130F	2	-	1/4	E, K, P
VHL 606 C>A	393 C>A	N131K	2	*	1/1	E, K, P, I
VHL 607 C>T	394 C>T	Q132X	2	*	1/2	E, K, P, I
VHL 620 T>G	407 T>G	F136C	2	*	3/4	E
VHL 665 T>C	452 T>C	I151T	2	-	1/10	E, C, K
VHL 666 C>G	453 C>G	I151M	2	*	1/1	C, K
VHL 676+2 T>C	463+2 T>C	Splice Defect	2	*	1/4	E, C, K, P

VHL 677-2 A>G	464-2 A>G	Splice Defect	3	*	1/6	E, C, K, P, I
VHL 679 T>A	466 T>A	Y156N	3	-	1/1	none
VHL 680 A>G	467 A>G	Y156C	3	*	7/11	C
VHL 694 C>T	481 C>T	R161X	3	*	2/29	E, C, K, P
VHL 695 G>A	482 G>A	R161Q	3	*	10/10	E, C, K, P
VHL 695 G>C	482 G>C	R161P	3	*	1/4	E, C, K, P, I
VHL 701 T>A	488 T>A	L163H	3	-	2/3	E, C, K, P, I
VHL 703 C>T	490 C>T	Q164X	3	*	1/4	E, C, K, P
VHL 709 G>T	496 G>T	V166F	3	*	1/1	E, C, P
VHL 712 C>T	499 C>T	R167W	3	*	20/37	E, C, K, P, I
VHL 713 G>A	500 G>A	R167Q	3	*	14/23	E, C, K, P, I
VHL 722 T>G	509 T>G	V170G	3	*	1/1	none
VHL 738 C>G	525 C>G	Y175X	3	*	1/1	E, C, P
VHL 746 T>A	533 T>A	L178Q	3	*	3/3	E, C, P
VHL 751 A>G	538 A>G	I180V	3	*	1/1	none
VHL 761 C>A	548 C>A	S183X	3	*	2/9	E, C, K, P, I
VHL 775 C>G	562 C>G	L188V	3	*	9/14	E, C
VHL 796 C>T	583 C>T	Q195X	3	*	3/6	E, C, K, P, I
VHL 806 T>A	593 T>A	L198Q	3	-	5/10	I
VHL 853 T>G	640 T>G	X214G	3	-	3/4	E, C
VHL Deletion Exon 1	VHL Deletion Exon 1	Deletion	1		1/16	E, C, K, P, I
VHL Deletion Exon 1+2	VHL Deletion Exon 1+2	Deletion	1+2		1/8	E, C, K, P
VHL Deletion Exon 2	VHL Deletion Exon 2	Deletion	2		1/11	E, C, K, P
VHL Deletion	VHL Deletion	Deletion	1-3		1/55	E, C, K, P, I

Exon 1-3	Exon 1-3					
VHL Deletion Exon 2+3	VHL Deletion Exon 2+3	Deletion	2+3			E, C, K, P
VHL Deletion Exon 3	VHL Deletion Exon 3	Deletion	3			E, C, K, P, I

Table 10: Mutations of the VHL gene that were identified in the Freiburg laboratory in patients with Pheochromocytoma.

Abbreviations for tumors or cysts in other organs: E=Eye tumor, C=Tumor in the central nervous system, K=Tumor in one kidney, P=Pancreas cysts, I=Islet cell tumors

\*Mutations that have been published on the internet.

The authors published a separate brochure for the VHLp.Y98H mutation in German.

The VHL mutations were published on the internet: [www.umd.be/VHL/](http://www.umd.be/VHL/).

Mutation	Amino acid	Exon	HGMD	LOVD	Localizations
SDHB c. 155 del C	S8PfsX2	1	-	+	Extra-adrenal, thorax, Glomus tumor
SDHB c. 183 del A	T17PfsX60	1	+	+	Glomus tumor
SDHB c. 213 C>T	R27X	2	+	+	Extra-adrenal, Glomus tumor
SDHB 221_224 dup CCAG	T31PfsX33	2	-	+	Adrenal
SDHB c. 270 C>G	R46G	2	+	+	Adrenal, extra-adrenal, thorax, Glomus tumor
SDHB c. 271 G>A	R46Q	2	+	+	Adrenal, Glomus tumor
SDHB c. 291 G>A	G53R	2	+	+	Adrenal
SDHB 300_304 del CCTCA	P56YfsX5	2	+	+	Extra-adrenal
SDHB c. 328 T>C	L65R	2	+	+	Adrenal, extra-adrenal
SDHB c. 394 T>C	L87S	3	+	+	Extra-adrenal
SDHB 402 C>T	R90X	3	+	+	Adrenal, extra-adrenal
SDHB c. 421-2 A>G	Splice site	4	+	+	Adrenal, extra-adrenal, thorax, Glomus tumor
SDHB c. 436 G>A	C101Y	4	+	+	Extra-adrenal
SDHB c. 462 A>C	T110P	4	+	+	Adrenal, Glomus tumor
SDHB c. 557+1 G>A	Splice site	4	+	+	Adrenal, Glomus tumor
SDHB c. 637 dup A	Q169AfsX10	5	-	-	Extra-adrenal
SDHB c. 675-2 A>G	Splice site	6	-	+	Extra-adrenal, Glomustumor
SDHB 708 T>C	C192R	6	+	+	Extra-adrenal
SDHB c. 709 G>A	C192Y	6	+	+	Extra-adrenal
SDHB 721 G>A	C196Y	6	+	+	Adrenal, extra-adrenal
SDHB c. 783 C>T	R217C	7	+	+	Adrenal, extra-adrenal
SDHB c. 822 C>T	R230C	7	+	+	Adrenal, extra-adrenal, Glomus tumor
SDHB c. 823 G>A	R230H	7	+	+	Extra-adrenal, Glomus tumor

SDHB 823 G>T	R230L	7	+	+	Glomus tumor
SDHB c. 859 G>A	R242H	7	+	+	Adrenal, Glomus tumor
SDHB c. 870 A>T	I246F	7	+	+	Glomus tumor
SDHB c. 881 C>A	C249X	7	+	+	Adrenal
SDHB c. 899+1 G>A	Splice site	7	+	+	Adrenal, extra-adrenal, Glomus tumor
SDHB Del Exon 1	Deletion	1	+	+	Adrenal, extraadrenal, Glomus tumor
SDHB Duplikation Exon 3	Duplication	3	+	+	Extra-adrenal, Glomus tumor

Table 11: Selected mutations of the SDHB gene that were identified in the Freiburg laboratory.

Mutations of the SDHx group were published on the internet: [www.umd.be/HGMD/](http://www.umd.be/HGMD/) or [www.umd.be/LOVD/](http://www.umd.be/LOVD/).

Locations: tumors are exclusively located in the autonomous nervous system

Mutation	Amino Acid	Exon	HGMD	LOVD	Localizations
SDHC c. 3 G>A	M1?	1	+	+	Glomus tumor
SDHC c. 23 dup A	H8QfsX12	2	+	+	Glomus tumor
SDHC c. 39 C>A	C13X	2	+	+	Glomus tumor
SDHC c. 43 C>T	R15X	2	+	+	Glomus tumor
SDHC c. 148 C>T	R50C	3	+	+	Glomus tumor
SDHC c. 173 T>C	I58T	3	+	+	Glomus tumor
SDHC c. 210 C>G	C70W	4	+	+	Glomus tumor
SDHC c. 214 C>T	R72C	4	+	+	Glomus tumor
SDHC c. 218 ins A	Splice site	4	+	+	Glomus tumor

Table 12: Selected mutations of the SDHC gene that were identified in the Freiburg laboratory.

Mutations of the SDHx group were published on the internet: [www.umd.be/HGMD/](http://www.umd.be/HGMD/) or [www.umd.be/LOVD/](http://www.umd.be/LOVD/).

Locations: tumors are exclusively located in the autonomous nervous system



Mutation	Amino Acid	Exon	HGMD	LOVD	Localizations
SDHD c. 2T>A	M1?	1	+	-	Glomus tumor
SDHD c. 14 G>A	W5X	1	+	+	Adrenal, extra-adrenal, thorax, Glomus tumor
SDHD c. 33 C>A	C11X	1	+	+	Adrenal, extra-adrenal, thorax, Glomus tumor
SDHD c. 36_37 del TG	A13Pfs X55	1	+	+	Adrenal, extra-adrenal, Glomus tumor
SDHD c. 49 c>T	R17X	1	+	+	Glomus tumor
SDHD c. 52+1 G>T	Splice site	1/2	-	-	Adrenal
SDHD c. 52+2T>G	Splice site	1/2	+	+	Adrenal, Glomus tumor
SDHD c. 53-2 A>G	Splice site	1/2	-	+	Glomus tumor
SDHD c. 112 C>T	R38X	2	+	+	Adrenal, extra-adrenal, thorax, Glomus tumor
SDHD c. 184^185 ins TC	A62Sfs X25	3	+	+	Glomus tumor
SDHD c. 209 G>T	R70M	3	+	+	
SDHD c. 242 C>T	P81L	3	+	+	Glomus tumor
SDHD c. 274 G>T	D92Y	3	+	+	Glomus tumor
SDHD c. 317 G>T	G106V	4	+	+	Adrenal, extra-adrenal, thorax, Glomus tumor
SDHD c. 337_340 del GACT	D113Mf sX21	4	+	+	Glomus tumor
SDHD c. 341 A>G	Y114C	4	+	+	Adrenal, Glomus tumor
SDHD c. 361 C>T	Q121X	4	+	+	Adrenal, extra-adrenal
SDHD c. 370 del G	A124Pfs X11	4	+	+	Glomus tumor
SDHD c. 441 del G	G148Af sX20	4	+	+	Adrenal, extra-adrenal, thorax, Glomus tumor
SDHD c. 443 G>T	G148V	4	+	+	Glomus tumor

SDHD Deletion Exon 1	Large deletions	1	+	-	Glomus tumor
SDHD Deletion Exon 3	Large deletions	3	+	-	Glomus tumor
SDHD Deletion Exon 3+4	Large deletions	3+4	+	-	Glomus tumor

Table 13: Selected mutations of the SDHD gene that were identified in the Freiburg laboratory.

Mutations of the SDHx group were published on the internet: [www.umd.be/HGMD/](http://www.umd.be/HGMD/) or [www.umd.be/LOVD/](http://www.umd.be/LOVD/).

Locations: tumors are exclusively located in the autonomous nervous system

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