Jeroen C. Jansen Paragangliomas of the Head and Neck

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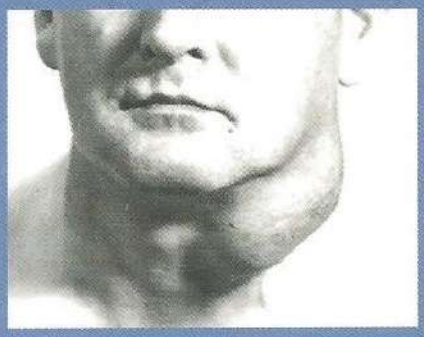
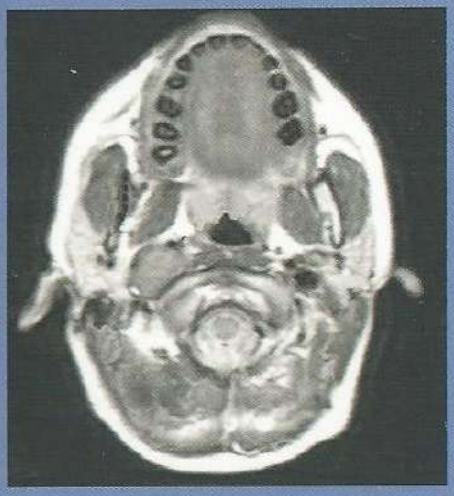


Clinical Implications of Growth Rate and Genetics



Jeroen C. Jansen

Paragangliomas of the Head and Neck

Clinical Implications of Growth Rate and Genetics

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Clinical Implications of Growth rate and Genetics

PROEFSCHRIFT

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in 1964

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	Dr. P. Devilee

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Introduction & Outline of the Thesis

This thesis is dedicated to rare neoplasms that I will refer to as *head and neck paragangliomas* but which are also commonly known as *glomus tumors* or *chemodectomas*. The Netherlands boasts a long history of research in head and neck paragangliomas. Since 1933 when Goekoop first described jugulotympanic paragangliomas, at least eight academic dissertations have been published at different Dutch universities. In the Leiden University Medical Center the research into head and neck paragangliomas started when Prof. Dr. P.H. Schmidt, former head of the ENT Department, initiated a study to compare the outcome of surgery with the natural course of paragangliomas. During this study Van der Mey made an inventory of the hereditary cases of head and neck paragangliomas and, coincidentally an extraordinary inheritance pattern was recognized. Since then a multidisciplinary research group -that includes human and clinical genetics, pathology, endocrinology, medical statistics, radiology, physiology, vascular surgery, neurosurgery and ENT- has been working on different aspects of head and neck paragangliomas. Collaborations with other institutes have been of great importance. The cooperation with the Departments of Genetics from Rotterdam University, Nijmegen University and Pittsburgh University have been particularly very stimulating. The Department of Surgery in the Utrecht Medical Center has provided a substantial number of tumors and blood samples.

After a concise review of the current knowledge of head and neck paragangliomas and the paraganglion system from which they arise, a series of studies are brought together in this thesis. While Chapter 2 describes the application of a new diagnostic tool, the main focus of these investigations is the natural course and the genetics of (hereditary) head and neck paragangliomas. The general discussion focuses on the clinical implications of these studies. The main topics and subsequent chapters are briefly introduced below.

Natural Course

The most important feature of any disease is the natural course of the illness. All treatment, whether surgical or medical, has unwanted side effects that must be weighed against the expected benefit. Most diseases are self limiting and are only treated to shorten the duration of the disease and to relieve symptoms. In these cases treatment with serious side effects is not acceptable. Other diseases invariably lead to death if untreated and warrant aggressive, potentially dangerous therapies. Many diseases, including paragangliomas, have a natural course that is variable and require great experience and knowledge to choose the optimal treatment strategy.
The natural course of head and neck paragangliomas is difficult to study. The disease is very rare and most publications report case histories rather than large series of patients. Experience has shown that head and neck paragangliomas generally are indolent tumors that become aggressive in some cases, leading to death due to intracranial extension or dissemination. If the treatment of head and neck paragangliomas was uncomplicated these tumors should be extirpated without delay. Unfortunately this is not the case. The proximity of the carotid artery and

cranial nerves makes surgery of head and neck paragangliomas difficult. Prior to the 1950's it had an operative mortality of approximately 30%. Since then the operative techniques and instruments have been improved, still considerable morbidity can be induced by the treatment of head and neck paragangliomas.
Before embarking on surgery the alternatives therefore must be carefully considered for each individual case. In the absence of studies describing the natural course of the disease, the decision to treat is made on the basis of the personal experience of the physician and the traditions of the institution.
Our goal is to provide quantitative data on the natural course of the disease in order to improve the complicated management of head and neck paragangliomas.
In 1992 van der Mey published his retrospectively gathered results and concluded that surgery of head and neck paragangliomas did not improve the life expectancy of the patients, but rather increased the prevalence of cranial nerve palsy in cases of vagal body paragangliomas and jugulotympanic paragangliomas.[142] This subsequently led to a more conservative management in our hospital and a 'wait and scan' policy was introduced. This made it possible to observe an increased number of patients and describe new aspects of the natural course of head and neck paragangliomas.

Genetics
In collaboration with the Human Genetics Department of Pittsburgh University we recently demonstrated mutations of the SDHD gene on chromosome 11q23, in hereditary paragangliomas.[18]
This breakthrough is the result of more than 10 years work of the Leiden Hereditary Paraganglioma Project. This project was started in 1989 when a comparative study of pedigrees from head and neck paraganglioma families led to the unexpected conclusion that the disease did not obey Mendelian rules of inheritance.[143] Van der Mey, together with Prof. Dr. J.J.P. van de Kamp (former head of the Department of Clinical Genetics: KGCL), observed a complete absence of the disease in the children of female carriers. Prof. Dr. C.J. Cornelisse (pathology) related this phenomenon to genomic imprinting, a parent specific expression of genes.
This made identification of the paraganglioma gene not only important for genetic counseling and for the knowledge of the biology of paragangliomas but also for (tumor) genetics in general, and made it possible to acquire funds from KWF and NWO.
The genetic analysis was carried out at the Department of Human- and Clinical Genetics of the LUMC (head: Prof. Dr. G.J. van Ommen) and led by Dr. P. Devilee. The first success was localization of a gene (PGL1) on chromosome 11q.[91] This was achieved in collaboration with the Clinical Genetic Department of the Rotterdam University (head: Prof. Dr. H. Galjaard).
The process of the further localization of the gene is described in Van Schothorst's thesis "Genetics of Hereditary Head and Neck Paragangliomas".[182] Extensive studies of the patient's pedigrees not only yielded new data on molecular genetics but also provided data on the genetic epidemiology of hereditary head and neck paraganglio-

mas in the Netherlands, which are presented in this thesis.

Outline of the thesis

Chapter 1 is an introduction to the paraganglionic system and paragangliomas of the head and neck.

Chapter 2 describes the experiences with color Doppler imaging (a non invasive method to demonstrate blood flow by means of ultrasound) of paragangliomas in the neck.

Chapter 3 of this thesis reports on the natural course of head and neck paragangliomas. It describes the growth rate of a series of 48 tumors. Since we have become more conservative in our indications for surgery, we have been able to estimate tumor volume using sequential radiological imaging. It was found that 81% of the paragangliomas had a doubling time of >3 years, supporting our 'wait and scan' policy.

Chapter 4 is concerned with the penetrance of hereditary paragangliomas, which is another aspect of the natural course of this disease. The age of onset, usually between 20 and 40 years, already shows that this penetrance is age dependent. Nevertheless we assumed that all people at risk for head and neck paragangliomas would eventually develop one or more paragangliomas. Our study in a large family with head and neck paragangliomas compared the clinical status with the genetic status. At the age of 75 years all persons at risk had developed a radiologically proven tumor whereas only 58% developed symptoms. Thus demonstrating again the indolent natural course of most head and neck paragangliomas, an important point for genetic counseling.

Chapter 5 of this thesis reports the confinement of this gene to a 2-cM interval on 11q22-q23. Genealogical study of the involved families proved to be very useful in this study.

Chapter 6 is another example of the successful cooperation with the Department of Human- and Clinical Genetics. The combination of genetic and genealogical research demonstrated that a single founder mutation was present in all 10 paraganglioma families from the Dutch province of *Zuid-Holland*. Involvement of an ancient mutation illustrates the fact that head and neck paragangliomas are not associated with reduced reproductive fitness and indicates a low mutation frequency of the PGL1 gene.

Chapter 7 can be regarded as a molecular genetic sequel of chapter 5 as -after PGL1 was identified as the SDHD-gene- it describes the founder mutations in SDHD encountered in familial and also notably in isolated cases of head and neck paragangliomas. It proves that we underestimate the prevalence of hereditary cases.

Chapter 8 specifically addresses the underestimation of the prevalence of hereditary head and neck paragangliomas. Hereditary head and neck paragangliomas are considered to occur less frequently than sporadic cases. Only 10% of the total number would be attributable to a genetic cause. However, there are several reasons why patients are not aware that the disease runs in the family. Whether due to the slow growth rate or genetic reasons, not all patients with head

and neck paragangliomas develop symptoms of their tumor. (Chapter 3 & 4) Moreover, if the mutation is inherited from the mother the penetrance is completely suppressed. (Chapter 5 & 7) This makes it more difficult to ascertain hereditary cases. In chapter 7 the presence of germ line mutations in isolated cases of head and neck paraganglioma is confirmed.
In order to estimate the prevalence of hereditary cases in a certain population we designed a simple statistical method. It is based on the assumption that all cases with multiple paragangliomas are inherited. In our series we estimate the prevalence of hereditary cases to be 78%. In a general population it would be ~40%. The difference could be attributed to the founder effect in our region. (Chapter 5) These results show that the concept of head and neck paragangliomas as being a sporadic disease that is occasionally inherited should be abandoned and that a hereditary etiology must be considered for every patient.
Chapter 9 finally reports our first experiences with genetic counseling based on predictive DNA diagnosis. This is an important issue because it links the laboratory experiments with clinical practice. Unlike some hereditary diseases that can be cured if the carrier is detected presymptomatically, genetic counseling cannot prevent hereditary paragangliomas. However, early detection of a tumor could be beneficial for the patient. Moreover family members that are not carriers of the mutation can be reassured. On the other hand genetic counseling and subsequent follow up might unnecessarily worry asymptomatic carriers who may never develop symptoms of their disease.

Chapter 1

The Paraganglion System and Head and Neck Paragangliomas

The section on the normal tissue from which paragangliomas arise is largely based on the comprehensive work of Zak and Lawson, who have meticulously organized and described all literature on the paraganglion system between 1743 and 1980.[217]

The section on paragangliomas is adapted from "The Management of Carotid Body Tumors"
AGL van der Mey, JC Jansen, JA Van Baalen. Otolaryngological Clinics of North America. In press.

Chapter 1

The Paraganglion System
and
Head and Neck Paragangliomas

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The paraganglion system

Adrenal and extra-adrenal chromaffin tissue

Paraganglions are cell-clusters of neuroectodermal origin that have a close relationship to the autonomic nervous system and have the ability to synthesize, store and secrete catecholamines (dopamine, norepinephrin and epinephrine). If present in sufficient quantity these compounds lead to a positive chromaffin reaction with chromates under the light microscope. Therefore the paraganglionic tissue is often said to be *chromaffin*, which is -as will be revealed- not always the case.[40]

The largest paraganglion is the adrenal medulla, a key organ in the sympathetic regulation. The word *paraganglia* however, is derived from the numerous chromaffin bodies present along the sympathetic chain, intimately associated with the ganglia. Moreover, other so-called extra-adrenal chromaffin paraganglions are present in the retroperitoneal region around the aorta, between the renal arteries and the aortic bifurcation. Situated here is the largest extra-adrenal portion of the system 'the organ of Zuckerkandl' (Figure 1-1).

During the fetal period the extra-adrenal chromaffin tissue is larger than the adrenal but involution of the former and increase of the latter reverses the ratio in the first years of life.[217] Finally chromaffin cell groups, that may be related to the paraganglions are present scattered through the peripheral nervous system.

Because of their cytochemical and ultrastructural features paraganglion cells are considered to be part of the APUD system (amine precursor uptake and decarboxylation).[164] Most organs contain cells from this system that includes other endocrine neuroectodermal cells like calcitonin producing C-cells of the thyroid, gastrin producing G cells in the stomach and insulin producing pancreatic islet cells.

The carotid body

Kohn recognized the similarity between sympathetic paraganglia and the carotid body.[104] This tiny structure, that is invariably present in the bifurcation of the carotid artery, may be difficult to distinguish histologically from small sympathetic paraganglia. The predominant cells in carotid bodies -type 1 or chief cells- produce and store catecholamines but usually not enough to give a positive chromaffin reaction. Ultrastructure studies yield sufficient similarities between carotid body chief cells and pheochromocytes of the adrenal medulla to support the theory of a common cell type.[72] Furthermore embryological studies as well as experimental evidence confirm the neuroectodermal origin of the chief cells.[105;121;164] However, there are several important differences between chromaffin paraganglia and the non-chromaffin carotid body.

1. The carotid body consists of two cell types (type 1: chief cells and type 2: sustentacular cells) that are organized in clusters called *'Zellballen'* and that are surrounded by a highly vascular stroma. Type 2 cells are extremely rare in the other paraganglia.[102]

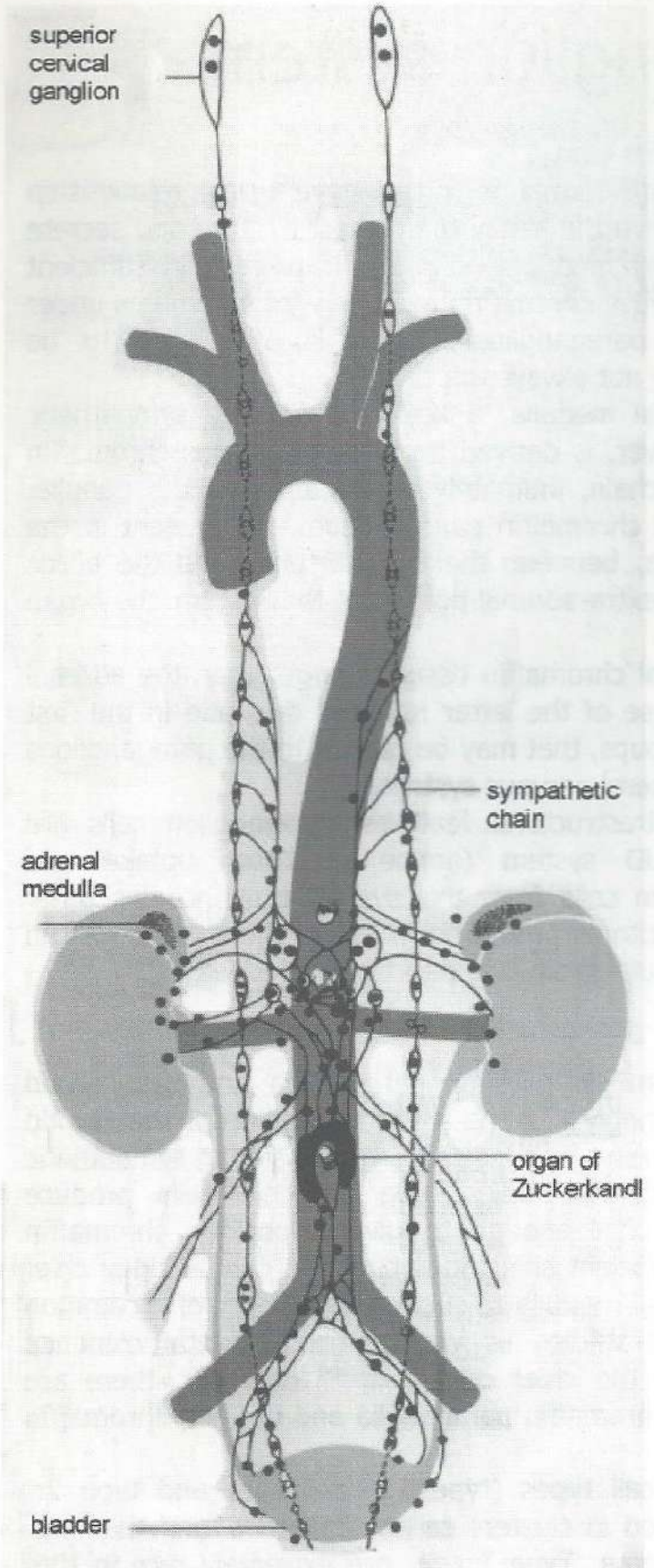

FIGURE 1-1
The aorticosympathetic or chromaffin paraganglion system.
The adrenal medulla is the major component of the system in the adult. During the fetal period the greatest amount of chromaffin tissue is extra-adrenal. Most of it is situated at the abdominal sympathetic outflow. The largest component of the extra-adrenal sympathetic system is the organ of Zuckerkandl. It consists of a pair of fused paraganglions situated at the origin of the inferior mesenteric artery. At the age of three the organ of Zuckerkandl reaches its largest size, after which it gradually decreases in size.
Original drawing from Coupland[40], modified by Zak and Lawson and the author.[217]

2. The carotid body contains mainly myelinated sensory nerve fibers of parasympathetic origin (through a branch of the glossopharyngeal nerve, Hering's nerve or carotid sinus nerve) and few fibers from the cervical sympathetic nerves or parasympathetic efferent secretomotor fibers.[45;102] The sympathetic paraganglia however, are predominantly innervated through preganglionic sympathetic fibers.[40]
3. The endothelium of chromaffin tissue is markedly fenestrated and in direct contact with the pheochromocytes whereas the endothelium is separated from the chief cells of the carotid body.[102]

It is likely that these differences indicate a functional difference between the two types of paraganglions. Whereas the role of the adrenal medulla is secretory, all physiological experiments point to a chemoreceptor function of the carotid body. Especially acute hypoxia, but also changes in CO_2 and pH lead to a chemoreflex ventilatory drive through the carotid body. Although many aspects of the peripheral chemoreceptor system are still unknown, the last decade has shown much progress in unveiling its physiological mechanisms.[50;168] It is has been firmly established that the chief cells (type I) are the sensory cells that respond to changes in arterial oxygen tension. The small amounts of catecholamines that are present within these cells probably serve as autoregulators of this response.

Other non-chromaffin paraganglions in the head and neck region

Following recognition of the carotid body as a paraganglion, other parasympathetically innervated paraganglions, some of which had been described before but had not been recognized as such, were subsequently identified in the head and neck region(Fig 1-2).

1. Vagal bodies at the nodose ganglion of the vagal nerve.[6]
2. Tympanic body at the promontory in the middle ear, innervated by the tympanic nerve of Jacobson, a branch of the glossopharyngeal nerve.[108]
3. Jugular body at the jugular foramen or bulb, innervated by the auricular branch of the vagal nerve (n. Arnoldi).[81]
4. Laryngeal bodies in the larynx innervated by the superior or inferior laryngeal nerve (branches of the vagal nerve).[208]
5. Cardioaortic bodies in the supracardiac region, innervated by the vagal nerve.[166]
6. The (very rare) occurrence of paragangliomas in the orbit is consistent with the observation of paraganglion cells in primates that are in close association with the parasympathetic fibers of the ciliary nerve.[28] The corresponding paraganglion however, has not sufficiently been demonstrated in humans.

All these organs are histologically identical with the carotid body. Only the cardioaortic bodies, found on along the aortic arch, have a proven chemosensory function comparable to that of the carotid body.[93]

Non-chromaffin paraganglia in other regions

In the lung and visceral organs (e.g. duodenum, bladder) minute paraganglionic bodies have been described, that may be related to the paraganglia in the head and neck, since a vagal innervation has been suggested.[26;75] Whether these structures represent the paraganglion system is still debated.

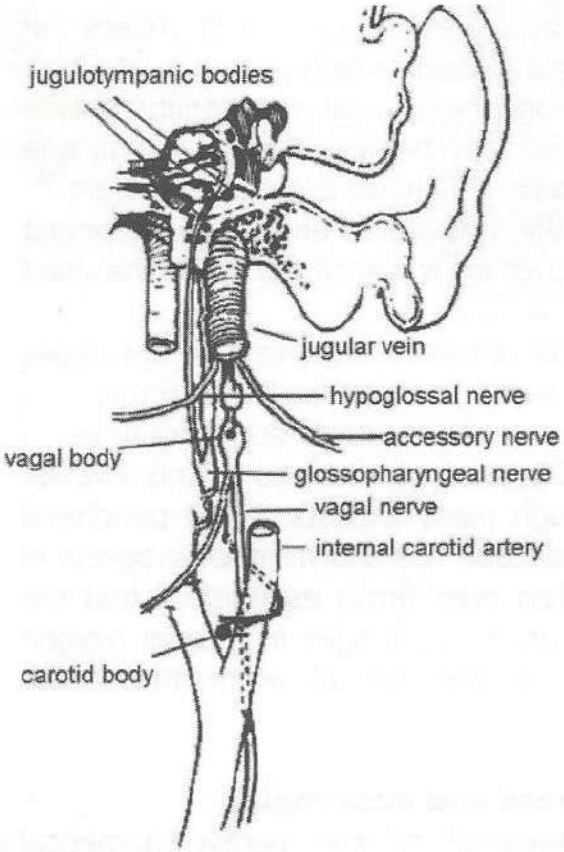

FIGURE 1-2
The extra-adrenal paraganglion system. Schematic drawing of the paraganglion system in the head and neck and its relation to the carotid arteries and lower cranial nerves. Note the association of the paraganglionic bodies with the (parasympathetic) branches of the vagal and glossopharyngeal nerve. Figure adapted from Zak and Lawson.[217]

Nomenclature

Although paraganglia in the true sense of the word are only present along the sympathetic chain, this term is more generally used. Paragangliomas are the tumors arising from paraganglions (except for tumors of the adrenal medulla that are called pheochromocytoma). In the international code of diseases (ICD-9-CM) these paragangliomas are grouped with little differentiation: code 227.5 benign neoplasm of carotid body (malignant 194.5); code 227.6 benign neoplasm of aortic body and other paraganglia (malignant 194.6). There is little consistency of nomenclature in the subgroup of paragangliomas to which this thesis is dedicated.
The associated paraganglions have been named according to histochemistry, presumed embryological origin, physiology, shape, nerve supply and anatomic site.

1. Histochemistry:
   The division between chromaffin and non-chromaffin paraganglia has been too heavily relied on. It became apparent that the difference in chromaffinity is but a quantitative difference and that both paraganglions contain catecholamines. Moreover, so called non-chromaffin paraganglia may occasionally be chromaffin, which in fact has led to the proposition of a paraganglion system by Kohn.[104]

2. Embryological origin:
   The paraganglia have been named presuming a glandular (ectodermal) origin or presuming a vascular (mesodermal) origin. The term *glomus* (tumor) is still used often and carries a possible risk of confusion with the highly vascular smooth muscle cell tumor (glomus) of Masson.[136] This is illustrated by the erroneous inclusion of the glomus coccygeum in the group of paraganglia in the official 'nomina anatomica' (codes 227.6 and 237.3).[57]
   Glenner and Grimley divide the paraganglia in four groups: branchiomeric paraganglia, intravagal paraganglia, aortico-sympathetic paraganglia and visceral autonomic paraganglia. Despite what the term might imply, the authors state that branchiomeric paraganglions are not embryologically related to the gill arches or to the branchial pouch mesoderm.[72] Rather the differentiation between intravagal paraganglia and branchiomeric paraganglia is made to stress the ill-explained fact that the vagal bodies are not intimately associated with arterial vessels.
3. Physiology
   The name suggested by Kjaergaard, *chemodecton* (and chemodectoma for the tumor) refers to the physiology of the carotid body and cardioaortic bodies.[102] The chemosensory function of paraganglion tissue at other locations still has to be proven. Therefore these bodies should be named differently.
4. Shape:
   Zak and Lawson prefer the term *body* accompanied by the anatomic location.[217] This is indeed very useful in the English literature but an international (Latin) translation -*corpus* or *corpusculum*- is not used, nor is the Dutch translation *lichaam*.
5. Nerve supply:
   The WHO classification uses the difference in innervation between the sympathetic paraganglia and those in the head and neck region, that are predominantly parasympathetically innervated. It comprises the following categories: pheochromocytomas, sympathetic paragangliomas, parasympathetic paragangliomas and paragangliomas not further specified.[213]
6. Anatomic site:
   Although we have adopted the term *head and neck paraganglions*, there is no complete confinement to the head and neck region since the cardioaortic bodies must be included. *Parasympathetic paraganglions*, as proposed by the WHO, is therefore probably the most correct term. Clinically, however, an anatomic description is more suitable and cardioaortic tumors are moreover extremely rare – we found 2 cases among 199 paraganglioma patients- thus indicating that parasympathetic paragangliomas can be regarded as a disease of the head and neck.

Head and Neck Paragangliomas

History

The most prominent paraganglion in the head and neck region is the carotid body, an organ with the size and shape of a grain of rice nested within the adventitia of the carotid bifurcation. Haller discovered the carotid body in 1742 (Figure 1-3).[201]

FIGURE 1-3
Albrecht von Haller (Bern 1708-1777). Swiss scientist, poet and politician. PhD at Leiden University (1727, promotor: H. Boerhaave). Professor of Anatomy, Botany and Surgery in Göttingen. Author of the "Elementa physiologiae corporis humani" (1757-66) and the famous poem "Die Alpen" (1729).
Painting by J.R. Studer (1745) downloaded from: http://www.haller.unibe.ch.

Almost 150 years later the first scientific report on carotid body tumors was made by Marchand who related the first, unsuccessful, resection of a carotid body tumor by Riegner in 1880.[132] A vagal body tumor, originating at the nodose ganglion of the vagal nerve, was described by Stout in 1935 but ignored until 1950 when Lattes rediscovered this clinical entity.[119;197] Lubbers was the first to describe a paraganglioma in the ear but only after the discovery of the jugular paraganglion by Guild was the nature of these tumors correctly interpreted by Rosenwasser.[81;128;177] Reports of paragangliomas in the thorax and larynx started in the 1950's, the latter even before the normal organ was described.[3;25;208] Later, histologically confirmed paragangliomas in the head and neck region, were described at various locations – including the orbit, the trachea, the esophagus and thyroid- that have not yet been shown to contain normal paraganglionic tissue.

Histology

Typically, paragangliomas in the head and neck region mimic the architecture of the normal tissue. They show an organoid pattern with cell nests (*Zellballen*) of epithelial cells (Type 1 or chief cells) surrounded by thin sustentacular cells (Type 2), that

separate the nests from the highly vascularized stroma, that is supported by a reticulin network. This stroma extends in a trabecular fashion from the periphery of the tumor where it is more firm and contains more collageneous fibers (Figure 1-4). In accordance with the theory that the chief cell is the primary tumor cell, this cell type dominates most tumors with less sustentacular cells and neuronal connections. Sometimes the cell nests are large and confluent and the chief cells have abundant cytoplasm (*adenoma type*), or the cells are more spindle shaped, mimicking mesenchymal cell clusters (*angioma type*).[39]
Head and neck paragangliomas at different locations have similar histological features. However, jugulotympanic paragangliomas tend to be less demarcated from the surrounding tissue and have more sinusoids between the *'Zellballen'*. Vagal body tumors often have more extended collageneous septa as compared to carotid body tumors.
Mitoses are rare in paragangliomas but may be seen in proven metastases that occasionally occur. Usually however, metastases of head and neck paraganglioma resemble the benign tumors.

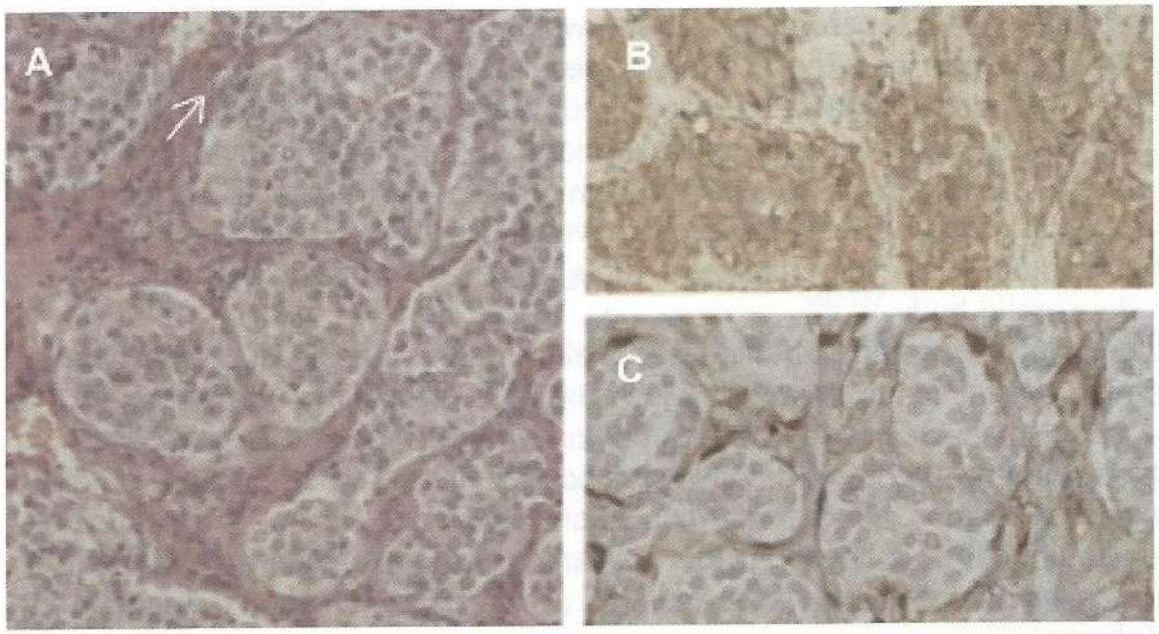

Figure 1-4 Histology of carotid body tumor
A. Typical appearance of large 'Zellballen' separated by vascularized (arrow) stroma. (HE-staining, 400x)
B. Chromogranin staining for type 1 cells.
C. S100 staining clearly delineates the elongated type 2 cells that surround the cell clusters.
(Courtesy of P.B. Douwes Dekker)

The differentiation of paragangliomas from others tumors can be difficult, especially if the patient history and the site of origin of the tumor is unknown to the examiner.[72] Therefore specific staining methods are imperative for the differentiation. The Grimelius argyrophilic stain was used to demonstrate neurosecretory granules in

paragangliomas.[115] Modern immunohistochemistry shows staining with *chromogranin* in chief cells, while sustentacular cells are *S100* positive.[140] Medullary thyroid carcinomas contain *calcitonin* and have characteristic amyloid deposits. Other thyroid carcinomas contain *thyreoglobulin*. Alveolar soft part sarcomas (ASPS) can easily be mistaken for paragangliomas if they do not contain characteristic diastase resistant, periodic acid-Schiff-positive crystals. Since most ASPS in the head and neck arise in the tongue or orbit of young adults, clinically this will be difficult only in the extremely rare case of an orbital paraganglioma. Indeed revision of 29 historic cases of orbital paraganglioma, in 16 cases led to reclassification of the tumor as ASPS.[4;96] Like (other) tumors arising from muscle tissue (leiomyosarcoma and rhabdomyosarcoma) *desmine* staining is almost always positive in ASPS. Granular cell tumors will stain diffusely with *S100* rather than have cell nests. Carcinoid tumors can be identified by demonstration of *5-hydroxy-tryptamine* (serotonin).[131] *Keratin* will disclose (adeno)carcinomas. In the rare case of an epitheloid Schwannoma the absence of cell nests and sustentacular cells will be conclusive.

Pathophysiology

Carotid body hyperplasia increasing with age was observed in high altitude dwellers in Peruvian studies that compared the size and weight of the carotid bodies between inhabitants of the Andes and those living at sea level.[5;178] A subsequent transformation of the hyperplastic carotid body into neoplastic disease was inferred by the observation of an increased prevalence of carotid body tumors in Peruvians living at high altitude.[87;174;178] Since others observed an irreversible desensitization of the chemoreflex response and a decreasing chemoventilatory drive during life in people born at high altitude, this hyperplasia is thought to compensate for the lack of function in the carotid body.[187;189]

Several authors have observed an increased weight of the combined carotid bodies of patients with chronic hypoxemia.[88;116] Given the high variation in normal weight of the carotid body (measured to be between 1 mg and 50 mg) and the influence of the vascular stroma on the carotid body size and weight, the value of these studies is questionable. A relation between cardiopulmonary disease and the occurrence of carotid body tumors as suggested by Saldana has never been confirmed.[178] Nissenblatt has suggested that hypoxia must be a congenital condition if it is related to paragangliomas.[157]

After resection of bilateral carotid body tumors, an invalidating labile blood pressure can be present in which the response to statuary change is not adequate. This is the result of injury to the sinus nerve involved with the pressure receptor in the carotid wall, and has no relationship to the carotid body physiology. Resection of carotid bodies has been shown to lead to a decrease in ventilatory response.[12;21;94;175] Whether or not the presence of head and neck paragangliomas leads to physiological changes in still unknown. Together with the Department of Anesthesiology and Physiology of the LUMC we are currently conducting a study on this subject.[41]

Tumor biology

The similarity between paragangliomas and the normal tissue has led several investigators to believe that these tumors are rather hyperplastic organs than true monoclonal expansions.[196] DNA ploidy studies have shown that aneuploidy in these tumors is not uncommon, indicating that neoplasia occurs. X-chromosome inactivation analysis has provided additional evidence for the monoclonal origin of paragangliomas.[48] Although some authors have suggested that DNA content can be predictive for clinical behavior most studies could not relate aneuploidy with local aggressive growth or the occurrence of metastases.[180] Recent studies with proliferation markers have conflicting results as well.[76] (P.B.Douwes Dekker data presented at the Dutch ENT society meeting, 2000) There has been some debate regarding the cell type that (eventually) exhibits clonal expansion. Histologically the type I cells usually increase in number. The loss of heterozygosity demonstrated in microdissected chief cells, proves that these cells must be regarded as the tumor cells.[183]

Genetics

Hereditary pattern

The first accounts of hereditary head and neck paragangliomas concerned carotid body tumors in siblings.[34;36] The report of 3 sisters with 'fibro-hemangiomas' by Goekoop is regarded as the first observation of hereditary jugulotympanic paraganglioma, although at that time the disease had not yet been described as such.[73] It was another Dutchman who re-diagnosed these cases and pointed to the possibility of developing hereditary head and neck paragangliomas at different sites in the head and neck region.[14] Kroll et al. concluded from their study in a large family with head and neck paragangliomas that the disease was transmitted as an autosomally dominant trait.[109] Ribet added to this that the penetrance was variable in the family he studied. Although sex-linked transmission was excluded, Hermann and van Baars et al. noticed an under-representation of affected offspring from female patients, but could not explain this observation.[90] In 1989 van der Mey et al. demonstrated that female carriers (affected or not) never had affected children and that the development of hereditary head and neck paragangliomas required transmission of the disease through a male carrier. Thus hereditary head and neck paragangliomas may skip multiple generations and make the ascertainment of a hereditary pattern difficult. Very recently a German family was described with head and neck paragangliomas (presumably) inherited in a normal autosomal dominant fashion.[155]

PGL genes

In 1992 the long arm of chromosome 11 was shown to harbor a gene (PGL1) associated with hereditary paragangliomas.[91] Simultaneously a second locus (PGL2) was assigned to region 11q13, on the same chromosome. Coincidentally, both studies involved families from the Netherlands. Only linkage to the PGL1 locus was found in other families. However, heterogeneity analysis confirmed the existence of

the PGL2 locus, although responsible only for 7% of the hereditary cases.[182]
Recently PGL1 has been shown to be identical with the SDHD gene that encodes for a protein of the oxidative pathway. SDHD (succinate-ubiquinone oxidoreductase subunit D) is a small part of cytochrome b558 of the mitochondrial respiratory chain complex II (Figure 1-5). In the Dutch founder families (chapter 6) a missense mutation that changes $ASP^{92} \rightarrow$ Tyr was found. Other missense mutations as well as nonsense mutations have been reported.[200]
Interestingly a German family with head and neck paragangliomas showed no link to PGL1 or PGL2 suggesting a third locus (PGL3).[155] In this family the disease seems not to be imprinted but displays a normal autosomal dominant inheritance pattern. A mutation in SDHC, another subunit within complex II, was recently demonstrated in this family.

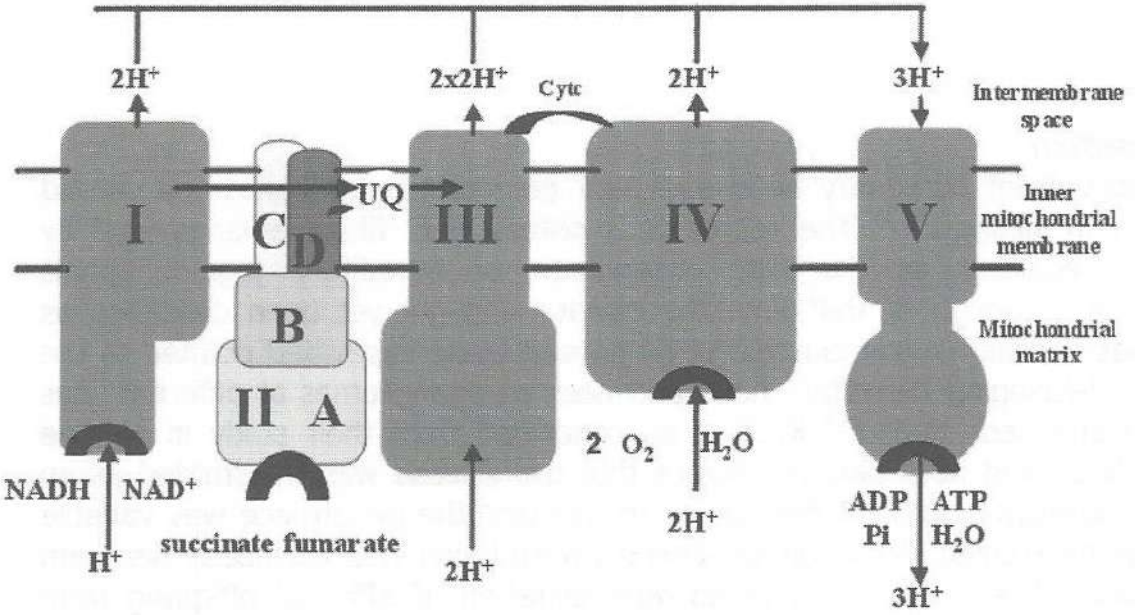

FIGURE 1-5
The five respiratory chain complexes in the mitochondrial membrane are designated with roman numbers. The second complex (succinate-ubiquinone oxidoreductase or succinate dehydrogenase) transfers an electron gained from succinate to the carrier ubiquinone (UQ) also known as co-enzym Q, that transports the electron to the next complex in the electron transport chain. SDHD is one of the four subunits of the second complex. The proton gradient (H^+) created in the inter-membranal space is used for production of the energy rich ATP. (Courtesy of dr. P. Taschner)

Genomic imprinting
The inheritance pattern of head and neck paragangliomas was explained by *genomic imprinting*. This non-Mendelian, epigenetic effect can be defined as a monoallelic and reversible, parent-specific mode of expression of mammalian genes.[13]
In other words, although the cells of an individual contain a maternal and a paternal copy of the involved gene, the copy of a specific parent is preferentially repressed.

In several diseases it has been shown that the promoter region of the imprinted gene, is made physically inaccessible by methylation. After removal of the methyl groups during gameto-genesis (or even during the course of life) the gene would be functional again. It is not likely that SDHD is imprinted in this fashion because only if mutant SDHD acts as an oncogene, the imprint mechanism could explain the absence of disease after female transmission. However, several studies have shown that in paraganglioma tissue the (presumably normal) maternal allele is lost (loss of heterozygosity, LOH), indicating that the normal gene prevents the development of head and neck paragangliomas.[18;183] This contradicts the theory of an oncogene and points towards an inactivated tumor suppressor gene. Then again it would seem strange that one copy of a tumor suppressor gene is imprinted in a normal person. Moreover one would expect a higher incidence of sporadic cases of head and neck paragangliomas since a single event could lead to complete loss of tumor suppression. Another interesting observation is the existence of two functional copies of the gene in lymphoblastoid cell lines of female carriers of the disease.[18] Thus, if SDHD is imprinted this will be tissue specific.
Preliminary explanations for genomic imprinting in head and neck paragangliomas include: The induction of imprinting by the mutation; involvement of a second, imprinted, gene; and the existence of an escape mechanism for the SDHD defect in oocytes of female carriers.[18;182;200]

Epidemiology

Incidence

Head and neck paragangliomas are very rare. In 1979 Batsakis estimated the number of published cases of carotid body tumors to be 500, jugulotympanic paragan-gliomas 300 and vagal body tumors 25.[16] Pooled data from Dutch pathological laboratories (PALGA) show an average incidence of 25 operated cases a year (1978-1992) corresponding to an incidence of 0.16 per 100,000 per year, given a population of 15 million people. Since not all paragangliomas are treated surgically the actual incidence in the Netherlands will be higher. However, it will not reach the incidence suggested by Bikhazi et al. of 1:30,000 in Caucasians for which, unfortunately, they do not give a reference.[23]

Sex

A female predominance has been implied especially with regard to jugulotympanic paragangliomas. The LUMC series contains 383 patients 222 of whom are female (55%). Among the patients with a jugulotympanic paraganglioma (n=144) 60% are female, confirming a slight predilection in women. However, the difference is so small it could reflect a more apprehensive approach towards neck masses and deafness in women. Surprisingly the series of Saldana in high altitude dwellers and Krause-Senties from Mexico show much more carotid body tumors in women.[107;178] Recently the study of Rodrigues-Cuevas confirmed this observation, finding a female preponderance of 8.3:1 and a very low prevalence of bilateral tumors.[174] He suggested that these tumors arising in high altitude dwellers form a distinct form of carotid

body paragangliomas. The differences between the sexes in chemoreflex development, especially at high altitude, may play a role in this intriguing observation.[101]

Race

Head and neck paragangliomas are encountered in all races. Most of the patients that have been described are Caucasian. In our series all but one of the patients are Caucasian. The exception is a woman from the Netherlands Antilla of whom the ancestors are unknown to us but likely include Caucasians as well as Africans.

Malignancy

Reports of malignant paragangliomas must always be met with scepticism because of their multicentric occurrence and the difficult histological differentiation between paraganglioma and malignant sarcoma. Nonetheless head and neck paragangliomas –especially jugulotympanic tumors- frequently exhibit infiltrative growth and sometimes develop regional or distant metastases.[16] The absence of any histological parameters that predict this locally aggressive behavior has led most authors to believe that the only true sign of malignancy in paragangliomas is metastasis.[207] The observed frequency of metastasis ranges from 0% to 20% but in most series is about 4%. [111;144;163] Laryngeal paragangliomas are thought to metastasize in 20% of the cases but occur so infrequently that large series are not available.

In our own series of 353 patients we had 5 malignant cases, three of whom had lymph node involvement in the neck and one had pulmonary metastases that, in the absence of symptoms, were not treated and have remained stationary for 17 years to date, indicating that the natural course of disease could remain so indolent. Others on the other hand, have described patients that rapidly deteriorated and died from carotid body tumor metastases.[135;207;214]

Association with other diseases

Several case histories suggest that head and neck paragangliomas often occur simultaneously with other neoplasms.[217] These include adrenal pheochromocytoma, neurofibroma, meningioma and other mostly neuro-endocrine tumors. Likewise head and neck paragangliomas have been reported in multiple endocrine neoplasia type II (Sipple's syndrome) and Carney's triad (gastric leiomyosarcoma, pulmonary chondroma and functional extra-adrenal paraganglioma).

Although one would expect mutations in the SDHD-gene to be expressed in a more general phenotype disturbance, our series does not support the idea that head and neck paragangliomas are often associated with other diseases. As an exception, pheochromocytoma was observed in 4 patients with germ line SDHD mutations. This may not be surprising since the adrenal medulla is a part of the paraganglion system itself, but other sympathetic paragangliomas were not present in this patient group. Several symptomless meningiomas were seen during screening of the skull base. This may be attributed to chance rather then to a syndromal sign.[98] Single cases of astrocytoma, pitituary adenoma, neurofibromatosis and Hürthle cell tumor, were observed.

Functional activity

Glomus tumors and pheochromocytomas form a class of neuroendocrine tumors with, as a common feature, the presence of numerous neurosecretory granules containing catecholamines. Occasionally head and neck paragangliomas cause a pheochromocytoma-like syndrome resulting from excessive tumor catecholamine production. In general the incidence of vasoactivity for head and neck paragangliomas has been estimated to be 1%.[71;120] The patient may present symptoms of headache, palpitation, labile hypertension and flushing. Although these cases are extremely rare, all patients should be evaluated with respect to elevated catecholamine production, because of the dangerous hemodynamic imbalance during and after surgery in unidentified cases. If the patient has symptoms or urine levels of catecholamine metabolites are elevated, MIBG scanning will reveal the anatomic origin that in some cases is a synchronous pheochromocytoma outside the head and neck region.[68] If a secreting paraganglioma is encountered, α-adrenergic blockage is necessary perioperatively.[66;186]

Clinical presentation

Age of onset

Head and neck paragangliomas have been recorded at all stages of life, from early childhood to old age.[38;89] The average age at which the diagnosis is established is ~45 years in most series. Typically there is a period of several years between the onset of symptoms and the diagnosis, probably due to the lack of symptoms and the low growth rate of the tumor.[116] In the LUMC series the age of onset ranges between 8 year and 76 year with an average of 37.2 year (Figure 1-6).

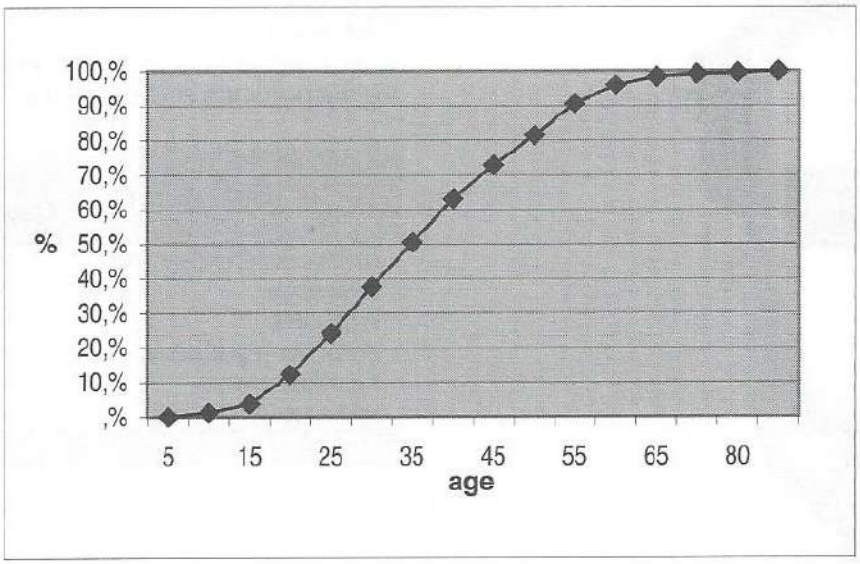

FIGURE 1-6 Age of onset (i.e. the age of the first symptoms) of 244 patients with head and neck paragangliomas.

Signs and symptoms

Head and neck paragangliomas present as indolent masses if located in the neck (Figure 1-7). The usually firm mass may be more fixed in a vertical rather than a lateral direction, due to fixation to the carotid vessels. The mass of the carotid body tumor is located in the carotid bifurcation, whereas vagal body tumors are found more cranial and often project into the lateral pharynx. Some tenderness may be present and even dysphagia if the mass is large. Jugulotympanic paragangliomas will cause symptoms in an early stage compared to their counterparts in the neck. Tumors of the tympanic paraganglion are initially confined to the middle ear cavity and cause pulsating tinnitus and conductive hearing loss. A reddish tumor may be seen behind the eardrum, that pulsates after application of pressure with a pneumatic otoscope (Brown's sign).[31] Due to secondary middle ear infection aural discharge may occur and spontaneous bleeding has been described. Involvement of the labyrinth leads to sensoneurinal hearing loss and vertigo. Paragangliomas of the jugular bulb usually project through the floor of the tympanic cavity and cause symptoms similar to tympanic tumors. However, if the tumor spreads caudally, the first signs may be involvement of the cranial nerves in the jugular foramen. Both tumors can affect the facial nerve and can cause intracranial complications. At the time of diagnosis differentiation between tympanic paragangliomas and jugular tumors is often difficult and without therapeutic consequences hence the combined nomenclature 'jugulotympanic paragangliomas' is used. In the LUMC series reviewed by van der Mey (1992) the inner ear and the facial nerve were most frequently affected (18% each) (Table 1-1).[142]

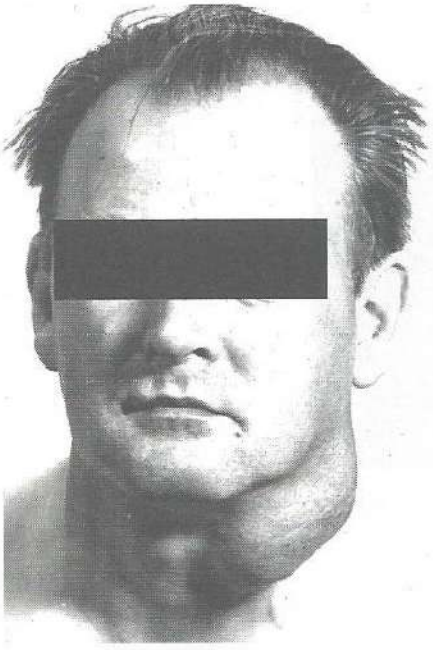

FIGURE 1-7
Patient with large carotid body and vagal body tumor on the left side.

TABLE 1-1
Cranial Nerve Involvement in Jugular Paragangliomas at Presentation

Nerve	% Affected at presentation
cochlea and cochlear nerve	18%
facial nerve	18%
vagal nerve	14%
glossopharyngeal nerve	10%
hypoglossal nerve	8%
spinal accessory nerve	4%

Head and neck paragangliomas usually progress slowly. In a series of 48 cases we observed growth in 60% during a mean follow up of 4.2 year. The estimated tumor doubling time ranged between 0.6 and 21.5 year. (Chapter 3)[100]

Diagnostic tests

The danger of head and neck paragangliomas is that this diagnosis is disregarded during the work up of a neck mass (or pharyngeal swelling in the case of vagal body tumors). Open biopsies of paragangliomas can be dangerous because of the extreme vascularity. If anticipating a lipoma or lymphoma this can lead to uncontrollable hemorrhage.

Clinically paragangliomas cannot be differentiated from other tumors in the neck. Differentiation from malignant disease, especially lymph node metastasis must be carried out swiftly. Less common differential diagnoses are neurogenic tumors or benign or malignant tumors arising from connective tissue. Finally benign lesions like branchial cleft cysts and carotid pseudo aneurysm must be taken into account.

Jugulotympanic paragangliomas are the most common middle ear tumors, therefore the otoscopic finding of a pulsating tumor in the middle ear is a valuable diagnostic clue.[97] Nevertheless it can be confused with polyps due to infectious disease as well as carcinoma of the middle ear and rare neoplasms like neurilemmoma and carcinoid. Meningiomas and Schwannomas with extraordinary lateral projection can be mistaken for middle ear tumors.

Magnetic resonance imaging

This is the most sensitive non-invasive tool for the diagnosis of head and neck paragangliomas (Figure 1-8).[22] With gadolinium enhancement intense staining will be observed in many cases accompanied by flow-voids within the larger vessels. High resolution is required to depict the smallest, clinically silent paragangliomas at other locations then the primary tumor. Using a head coil both plain and Gadolinium enhanced T_1 and T_2 weighted images should be made with small intervals (5 mm with a 0.5 interslice gap in our institution). In addition, 3D time of flight MR angiography sequences can be performed that allow scanning with a slice thickness of 1.5 mm. With this technique the sensitivity of MRI imaging for head and neck paragangliomas has increased from 80% to more than 90%. (R. van den Berg, personal communication) The tumor can be located within the carotid space (carotid body tumors and vagal body tumors) or in the jugular foramen. Vagal body tumors usually cause displacement of the internal carotid artery and usually do not extend to the carotid bifurcation it self. Once the diagnosis has been made, follow up imaging can be done with 3-D time of flight sequences alone in order to safe scan time.

Angiography

The extremely well vascularized paragangliomas are beautifully depicted with four vessel digital subtraction angiography (Figure 1-9). Differential diagnoses include mesenchymal tumors, Schwannoma, medullary thyroid carcinoma and hemangioma. Angiography is still superior to magnetic resonance angiography in identifying the

nutrient vessels of the tumor. Super selective angiography allows preoperative embolization of the tumor although this may be of little use in carotid body tumors.[51;126;181;192;206] If sacrifice of the internal carotid artery is anticipated angiography can be combined with balloon occlusion of the carotids to evaluate the patency of Willis' circle. Unfortunately, angiography does not inform us about the relationship with surrounding tissues and, being an invasive test, it bears more risk than other diagnostic tools, especially MRI.

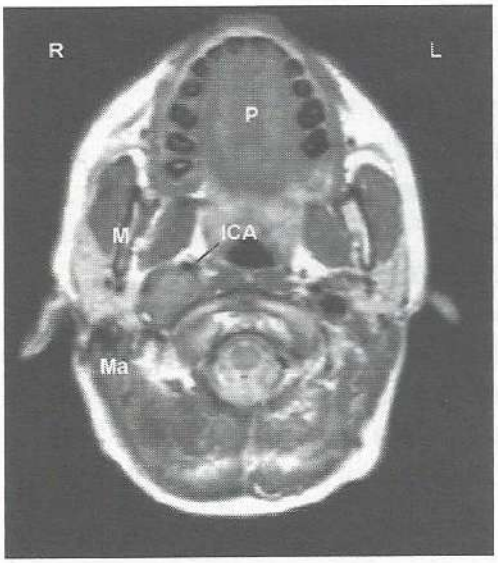

FIGURE 1-8
Transversal magnetic resonance image of a vagal body tumor at the right side, positioned medially to the mandible (M) mastoid tip (Ma) and lateroposterior to the internal carotid artery (ICA). Palate (P)

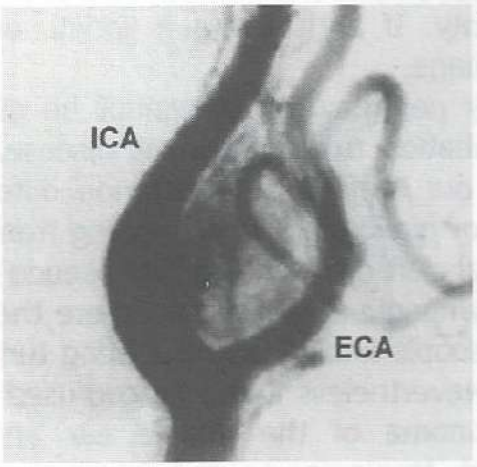

FIGURE 1-9
Angiography of a carotid body tumor.
The internal carotid artery (ICA) and external carotid artery (ECA) are displaced by a hypervascular tumor yielding a characteristic bulb like configuration.

Computed tomography

Computed tomography with intravenous contrast enhancement has been used for the diagnosis of head and neck paragangliomas but has proven to be less sensitive than MR-imaging (Figure 1-10).[103] As a complementary tool to evaluate bone erosion in temporal bone paragangliomas CT is however valuable.

Ultrasound

Ultrasound is not the best diagnostic instrument for paragangliomas as the presence of bone at the base of skull makes it impossible to examine this area. It is however the first line of imaging in the work up of neck masses. With conventional B-mode imaging paragangliomas cannot be differentiated from other solid tumors in the neck. With the use of superimposed Doppler flow information (Color Doppler Imaging, Duplex Scanning) high velocity intratumoral blood flow and splaying of the carotid bifurcation can be demonstrated (Figure 1-11, Chapter 2).[47] Ultrasound can also be helpful in performing a fine needle aspiration biopsy that might be useful in the differential diagnosis, especially between paragangliomas and squamous cell

carcinoma (see below).

Scintigraphy

The use of radioactive labeled compounds can be used for screening of paragangliomas. MIBG-scan (using I-123 labeled metaiodobenzylguanidine) can be used to detect hormonally active paragangliomas.[68] Uptake is also observed in neuroblastomas, carcinoids and medullary carcinomas of the thyroid.[191;205]
SMS scintigraphy, which detects somastatin receptors in a variety of tumors, has a much higher sensitivity than MIBG-scintigraphy and can be used to screen patients with head and neck paraganglioma for multicentricity.[112]

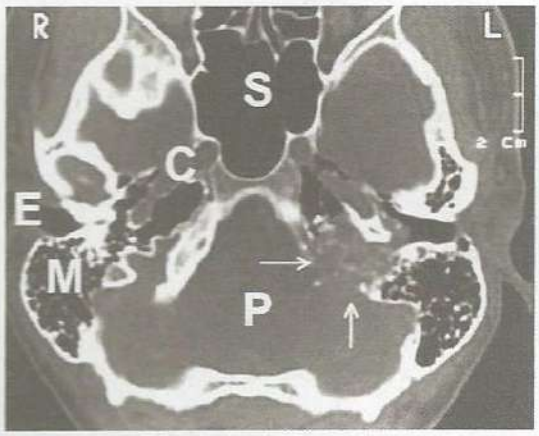

FIGURE 1-10
CT-image of a jugulotympanic tumor in the left (L) jugular foramen (arrows). The very well aerated petrosal bone is clearly eroded. The tumor extends into the middle ear, leaving the mastoid intact. The relation to the posterior fossa contents however, is unclear. E=external ear, M=mastoid, C=carotid artery, S=sphenoid sinus, P=posterior fossa.

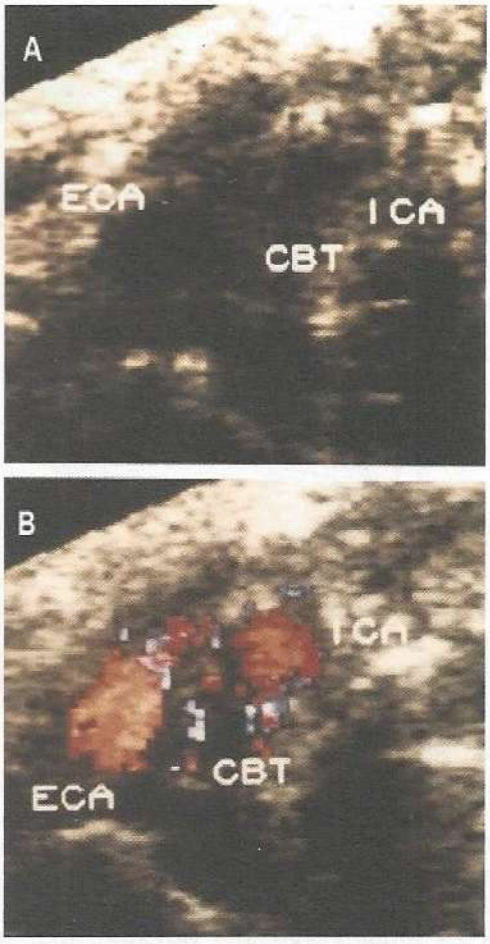

FIGURE 1-11
A) B-mode ultrasound image of a carotid body tumor (CBT). An echo poor lesion is present between the internal carotid artery (ICA) and external carotid artery (ECA).
B) Color Doppler image of the same CBT shows the blood flow in the carotids as well as in the tumor and facilitates the diagnosis.

Biopsy and Cytology

The clinical and radiological findings in CBT are thus pathognomonic and there is rarely any controversy concerning the need for biopsy to establish the diagnosis. An incisional biopsy is not advisable because of the risk of uncontrollable hemorrhage, injury of adjacent neurovascular structures and fibrosis, which complicates subsequent extirpation. Ultrasound guided fine needle aspiration biopsy can be helpful when the radiological diagnosis is unclear.[42;99;116] In these cases metastases of squamous cell carcinoma or thyroid carcinoma must be ruled out initially.

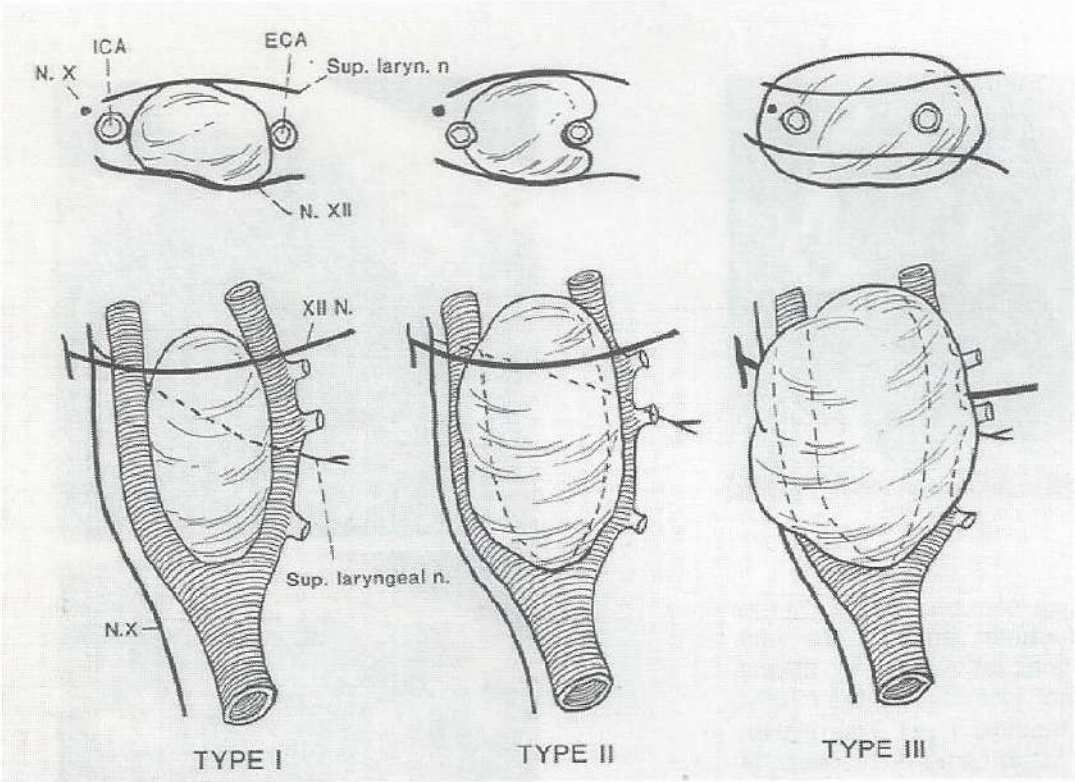

FIGURE 1-12
Classification of carotid body tumors according to Shamblin. Peroperatively the relation of the tumor to the carotid arteries (ICA and ECA), the vagal nerve (N.X) its superior laryngeal branch and the hypoglossal nerve (N.XII) are observed which gives an indication of difficulty of resection. Today the relation of the tumor to the carotid arteries can be established pre-operatively, but, in the absence of palsy, the relation to the nerves remains uncertain. (Adepted from Shamblin et al.)[188]

Classification

Carotid body tumors are classified according to Shamblin et al. (Figure 1-12).[188] This classification is indicative for the difficulty of resection but unfortunately has to be made intra-operatively. Type I tumors are confined to the carotid bifurcation. Type II includes tumors adherent to or partially surrounding the vessels. Type III CBT completely encase the carotids and reach the hypoglossal nerve. Most CBT (70 % in the Mayo-clinic series) are designated as type II and III and are considered to have increased risk of injury to the carotid vessels and cranial nerves during

resection.[83] This series also showed that the size of the tumor correlated better to the complication rate than did the Shamblin classification. Since tumor size can readily be established pre-operatively, authors describing treatment results should provide these data.

Vagal body tumors have not yet been subject to classification. However, important features that complicate surgery are a close relation to the base of skull and adherence to the carotid arteries.

Jugulotympanic paragangliomas are usually classified according to Fisch (Figure 1-13).[60]

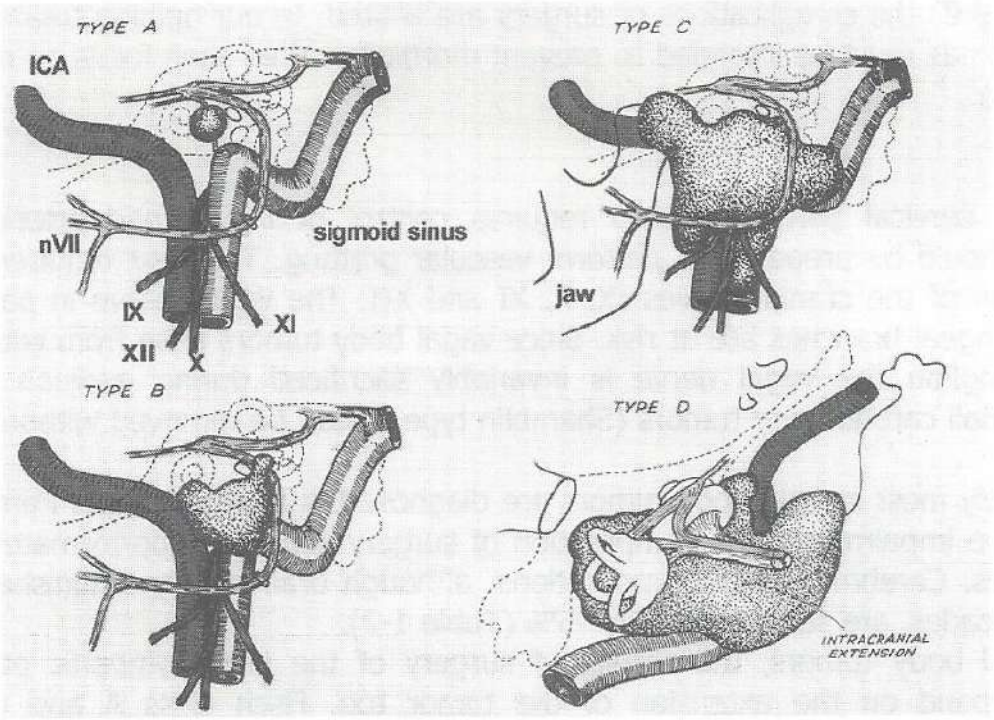

FIGURE 1-13

Classification of jugulotympanic paragangliomas according to Fisch.

Type A: Tumor confined to the tympanum and arising from the promontory without evidence of bone erosion.

Type B: Tumors involving the tympanum with or without mastoid involvement, but always arising from the hypotympanic region. The cortical bone over the jugular bulb must be intact.

Type C: Tumors eroding the bone over the jugular bulb. The tumor may extend into and destroy the bone of the infralabyrinthine, retrolabyrinthine, and apical compartments of the temporal bone.

- C1: Tumors involving the foramen caroticum.
- C2: Tumors involving the vertical segment of the carotid canal.
- C3: Tumors involving the horizontal segment of the carotid canal.
- C4: Tumors extending to the ipsilateral foramen lacerum and cavernous sinus.

Type D: Tumors with intracranial extension. Extradural and intradural extension are distinguished by the suffix "e" and "i" respectively.

- De1: Tumors with intracranial extradural extension up to 2 cm.
- De2: Tumors with intracranial extradural extension > 2 cm.
- Di1: Tumors with intracranial intradural extension up to 2 cm.
- Di2: Tumors with intracranial extradural extension >2 cm.
- Di3: Tumors with inoperable intracranial extension.

Treatment

The natural course of head and neck paragangliomas offers the opportunity to carefully contemplate the best treatment strategy. Some favor aggressive surgical excision given the risk of cranial nerve impairment due to progressive tumor growth others consider the risk of malignant degeneration when deciding to surgery.[149;162] We regard head and neck paragangliomas as benign tumors with an extremely indolent growth pattern. In any case it should be recognized that surgical therapy is often associated with neurovascular complications.[142;144;154;163] Only in surgery of carotid body tumors confined to the carotid bifurcation and tympanic paragangliomas (Class A and B) the complications of surgery are limited. In our opinion treatment of paragangliomas must be intended to prevent morbidity rather than focus on removal of the lesion.

Surgery

Surgery of cervical paragangliomas requires control of the carotid arteries and surgeons should be prepared to perform vascular grafting. The next requirement is identification of the cranial nerves IX, X, XI and XII. The vagus nerve in particular and its laryngeal branches are at risk. Since vagal body tumors arise from within the nodose ganglion the vagal nerve is invariably sacrificed during excision of the tumor.[43] Small carotid body tumors (Shamblin type 1) can be removed without much trouble.

Unfortunately most carotid body tumors are diagnosed in a larger stage. Permanent cranial nerve impairment as a complication of surgery occurs in approximately 20% of the cases. Cerebrovascular complications, although dramatically diminished over the past decades, are still reported in ~5% (Table 1-2).

Like carotid body tumors, the results of surgery of the jugulotympanic paragangliomas depend on the extension of the tumor too. Fisch class A and class B paragangliomas, confined to the middle ear and mastoid may be removed through a classic post-auricular approach with preservation of the hearing and cranial nerves, although the facial nerve is always at risk. Fisch class C tumors require control over the internal carotid artery and internal jugular vein. Inferiorly vascular control is possible in the neck but superiorly the petrosal part of the internal carotid artery has to be exposed. Anterior deflection of the facial nerve as described by Fisch (infra temporal fossa approach) provides exposure of the jugular foramen and the carotid artery bony canal. If sacrifice of the carotid artery is anticipated a balloon occlusion test should be performed preoperatively. Depending on the extension of the tumor the infratemporal fossa approach can be extended anteriorly (deflecting the zygoma and mandible) or posteriorly through the labyrinth into the posterior fossa. Most complications occur within the jugular foramen where cranial nerves IX, X and XI must be carefully dissected from the tumor (Table 1-3).

Radiotherapy

Reports of radiotherapy for head and neck paragangliomas show good results in local tumor control, although the definition of successful treatment is difficult in such

TABLE 1-2. Results of Surgery for Carotid Body Tumors

Author	Year	Period	Patients	Age	Female	Multiple	Familial	Malignant	†	CVA	CN palsy
Asperen[7]	1981	1958-79	42	41	45%	21%	33%	5%			21%
Rosen[176]	1981	1955-80	27		48%	15%	7%	4%	4%	7%	30%
Lees[122]	1981	1922-78	39	49	54%		5%			5%	
Parry[163]	1982	?-1977	222	45	66%	17%	7%	4%			
Krupski[110]	1982	1963-81	19	49	68%	5%	5%	20%	0%	20%	47%
Meyer[144]	1986	1975-84	13	48	77%		8%	0%			39%
Nora[158]	1988	1965-88	55	52	71%	24%	15%	2%	2%		21%
Hallett[83]	1988	1935-85	139	52							40%
McPherson[139]	1989	1975-87	25	47	64%	16%			0%	0%	16%
Kraus[106]	1990	1979-87	15	45	73%	47%					20%
Williams[214]	1992	1956-90	30	56	66%	13%		10%		3%	7%
Liapis[125]	1995	1975-93	14	46	64%				0%	0%	0%
Netterville[154]	1995	1986-94	30	42	57%	53%	53%	7%			20%
Mitchell[149]	1996	?	14	54	64%			6%	6%		12%
Litle[126]	1996	1984-94	21	46	64%					5%	48%
Gardner[64]	1996	1986-95	11	40	73%		55%				0%
Muhm[151]	1997	1962-95	24	51	58%		13%	4%	0%	4%	16%
Westerband[212]	1998	1981-97	31	48	52%	3%		3%		6%	13%
Bastounis[15]	1999	1975-95	17	45	65%		0%	0%	6%		
average				47.67					2.35%	5%	26%

If data is not available in the reference the field is left blank.
† =operative mortality

TABLE 1-3 Results of Surgery for Jugulotympanic Paragangliomas.

Author	Publ. year	Patients	Age	VII pre	VII post	IX pre	IX post	X pre	X post	XI pre	XI post	XII pre	XII post	Incomplete resection	†	Meningitis
Spector[193]	1973	45			16%									22%	0%	
Gardner[63]	1977	10	42	30%	100%	10%	10%	10%	10%	10%	10%	20%	10%	30%	10%	10%
Glasscock[70]	1979	11	29,4	25%			18%		27%		9%			9%	0%	
vd Mey[142]	1992	52		18%	33%	10%	20%	14%	26%	4%	8%	8%	16%	50%		
Green[77]	1994	52	47,7	12%	25%	23%	48%	34%	46%	17%	44%	19%	26%	15%	0%	4%

Hearing loss is omitted from the table due to the inconsistent reporting of this parameter.
If data is not available in the reference, the field is left blank.

an indolent neoplasm.[130]
Morfit (1967) doubted whether any patient was ever cured by irradiation and believed that claims of control or regression were related to the natural history of the tumor.[150] Relatively low doses of radiation are enough to induce substantial sclerosis and fibrosis possibly preventing tumor growth.[137] The optimum dose is still under discussion but many authors have reported excellent response with minimal side effects with doses of 40 to 50 Gray using conventional fractionation.[30;44] The tumor itself may not be radiosensitive and some have reported re-growth of initially 'regressed' paragangliomas.[188;204;207] In a histopathological study, Hawthorne and Fisch (1988) showed that tumor behavior after irradiation is unpredictable and that it should be reserved for the elderly and those in poor health with the aim of slowing down local tumor growth.[86] Perhaps the greatest drawback of radiotherapy is the surrounding fibrosis that greatly complicates secondary surgery. In young patients the risk of radiation-induced malignancies must be recognized.
The first reports of Gamma Knife and stereotactic radiotherapy for jugulotympanic paragangliomas are comparable to the results of conventional radiotherapy but lack the long term follow up which is obligatory in these patients.[55;61]

Complications and rehabilitation
Since the risk of cerebrovascular complications has in recent years been minimized, the most important complication of paraganglioma surgery is cranial nerve palsy. Though not mentioned in some studies the superior laryngeal nerve is possibly most frequently injured in surgery of cervical paragangliomas. It supplies the sensory innervation of the larynx and the cricothyroid muscle that makes falsetto voice possible. Although loss of the sensory function of the hemi-larynx may lead to some degree of aspiration, an isolated palsy of the superior laryngeal nerve requires no additional rehabilitation. Damage to the entire vagal nerve, within the jugular foramen or in the neck, adds a vocal cord paralysis to the previously mentioned sensory denervation. Although some degree of voice weakness invariably persists, the initially debilitating hoarseness and aspiration, gradually improves due to compensatory movement of the contralateral vocal cord. Instruction and exercise with a trained speech therapist will be helpful. If after an arbitrary 6-month period an insufficient glottic closure persists (possibly due to a difference in height between both vocal cords) phono-surgical intervention is possible. In order to prevent overcorrection we prefer the Isshiki method because of its controlled medialisation.
The glossopharyngeal nerve and accessory nerve run with the vagal nerve through the jugular foramen and are equally at risk. The hypoglossal nerve coming from its own foramen can usually be left intact unless it is infiltrated by the tumor. Its ansa cervicalis, running over the anterior wall of the internal jugular vein is more frequently injured which fortunately has little impact on the patient. If possible, end to end anastomosis of a divided hypoglossal nerve is worthwhile. If the excised part of the nerve is not too long interposition of the greater auricular nerve can be considered. Given the slurring and swallowing impairment of a unilaterally paralyzed tongue this effort must be made. The same holds for the more posteriorly running

accessory nerve. If the nerve is injured anteriorly from the sternocleidomastoid muscle the cervical contribution to this nerve will usually compensate for the loss. If not, the motor loss is not very debilitating but many patients complain of shoulder pain postoperatively. Therefore reconstruction if feasible should be attempted.
Like hypoglossal paresis, isolated glossopharyngeal lesions that result in sensory loss of the pharyngeal wall and dysfunction of the soft palate do not have a very detrimental effect on swallowing and speech. Because all of the involved cranial nerves are of functional importance for swallowing combined lesions, that often occur, are far more debilitating. Perhaps the most invalidating aspect is that those serious swallowing and speech disorders, which arise directly postoperatively and with which the patient will struggle to establish any kind of compensation, differ from the gradual effects which occur due to tumor involvement, in which case better recovery may be expected.

Concluding remarks

The paraganglion system consists of embryologically related cells that all have the capability to produce catecholamines. The parasympathetic innervation and (tentative) chemoreceptive function distinguishes a subgroup of paraganglions, that are confined to the head and neck region and upper thoracic cavity. The multicentric occurrence of paragangliomas confined to the head and neck region, confirms the existence of a division within the paraganglion system. On the other hand the occasionally associated adrenal pheochromocytoma proves that the extra-adrenal paraganglions are related to the adrenal medulla. *Parasympathetic paraganglions*, as proposed by the WHO, is therefore probably the most correct term for this subgroup of paraganglions and paragangliomas. Although much insight has been gained regarding the physiology of carotid bodies, the function of vagal body tumors (not intimately related to vascular structures) and jugulotympanic paragangliomas (located close to the venous drainage of the head) remains unclear. Not only the anatomic location but also the loss of ventilatory response after resection of the carotid bodies (keeping the other locations intact), suggest a functional difference between these paraganglions.
The identification of SDHD mutations in hereditary cases of head and neck paragangliomas has created new possibilities for studying the tumor biology. As SDHD plays a role in the cell's energy supply it is tempting to suggest that mutations in this system lead to hypo-function of the chief cells, which in turn might give a proliferation stimulus. Some evidence for this is the extraordinary number of mitochondria in paragangliomas and the upregulation of certain proteins functionally related to SDHD.[78] That such a stimulus would first result in hyperplasia of the paraganglionic body is not unlikely, providing support for the hyperplasia-neoplasia concept proposed by Heath. That a similar stimulus might be provided by hypoxia is conceivable, although the absence of paragangliomas in patients with chronic pulmonary or cardiac diseases, suggests that other factors are involved. Age is possibly one of these factors as the ventilation response changes dramatically soon after birth. In rats these changes depend also on the sex of the animal, which in turn

is interesting regarding the inheritance of head and neck paragangliomas.
Genomic imprinting is probably the most intriguing phenomenon of hereditary head and neck paragangliomas. The theory of an alternative pathway activated in the mitochondria of oocytes of SDHD mutation carriers is an attractive one to explain the absence of disease after maternal transmission. The existence of such an escape mechanism could also explain the very moderate phenotype (restricted to paragangliomas) that results from dysfunction of such a generally important system as the cytochrome-oxidase complexes.
The tumor biology of head and neck paragangliomas shows that behavior of neoplasms is not a simple concept of benign versus malignant. The histological criteria for malignancy (e.g. mitoses and infiltrative growth) seem to be unhelpful in predicting the natural course of these tumors. Incapacitating morbidity and even death can be caused by expansively growing paragangliomas, while metastases (regional or distant) can be very indolent and harmless. Therefore it is unnecessary to propose surgery for every head and neck paraganglioma for the reason that the tumor might be malignant.
If a disease is treated to prevent complications one must be able to predict the natural course of the disease. In chapter 3 and 4 of this thesis aspects of the natural course of head and neck paragangliomas are described. One of the gravest sequelae of these tumors is bilateral cranial nerve damage, which - very likely - only occurs, in hereditary cases. Hereditary cases may not however be as rare as many think (Chapter 9). It is therefore important to recognize the mutations causing this disease (Chapter 5, 6, 7). With the advent of DNA tests for presymptomatic carriership for head and neck paragangliomas new opportunities but also new problems have arisen for the patients, their family and the counselor (Chapter 8).

Chapter 2

Color Doppler Imaging of Paragangliomas in the Neck

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Chapter 2

Color Doppler Imaging of Paraganglioma in the Neck

Introduction

Paragangliomas are rare, usually benign tumors that arise from the extra-adrenal paraganglionic tissue, embryologically derived from the neural crest. The carotid body tumor is the most common paraganglioma and is typically found in the carotid bifurcation. Vagal body tumors are less common paragangliomas that arise near the vagal nerve, usually at the nodose ganglion. The presentation of both tumors is a slowly enlarging lateral neck mass and eventually cranial nerve palsy.[113;142]
Diagnostic imaging of paragangliomas relies mainly on the hypervascular nature of the glomus tissue (Figure 2-1).[103] The contribution of conventional B-mode ultrasound in the diagnosis of carotid body tumors, is limited to the accurate location of a solid tumor within the carotid bifurcation.[11] Sonographic diagnosis of vagal body tumors is hampered since their origin may be anywhere along the vagal nerve.[24]
Color Doppler Imaging has made image directed demonstration of tumor blood flow possible.[194] Therefore, Color Doppler imaging may be useful in the diagnosis of paragangliomas.[159] We present the results of color Doppler sonographic examinations performed on 17 patients who had a total of 25 previously diagnosed paragangliomas in the neck.

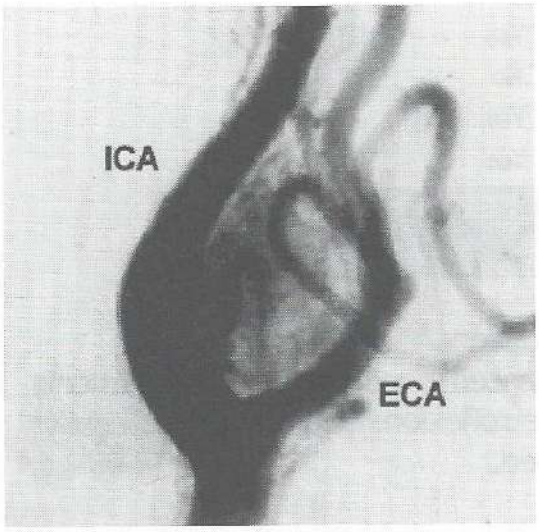

FIGURE 2-1
Angiography of a carotid body tumor. The internal carotid artery (ICA) and external carotid artery (ECA) are displaced by a hypervascular tumor yielding a characteristic bulb like configuration.

Patients and methods

Seventeen patients, 9 man and 8 women, between the ages of 26 and 69 years, underwent B-mode and color Doppler imaging (Table 2-1). Informed consent was obtained from all patients. Fourteen patients had a family history of paragangliomas, and in 13 patients a paraganglioma had had a paraganglioma resected in the past. Previously performed diagnostic imaging including angiography (1 patient), magnetic resonance imaging (6 patients), computed tomography (1 patient) or a combination of these techniques (9 patients), showed 25 cervical paragangliomas: 14 carotid body tumors and 11 vagal body tumors. Because we advocate a wait-and-see policy in glomus tumors, especially when they are multicentric, the diagnosis of none of the current tumors was confirmed by surgery.
B-mode and color Doppler imaging was performed with a Philips Angiodynograph

TABLE 2-1 Patient Characteristics

Patient	Age (years)	Sex	Hereditary	Previous Histology	Previous Imaging
1	53	F	Yes	Yes	CT, A, MRI
2	44	M	Yes	Yes	CT, MRI
3	35	M	Yes	Yes	CT, A, MRI
4	26	F	Yes	Yes	MRI
5	34	M	Yes	Yes	CT, A, MRI
6	28	M	Yes	No	A, MRI
7	35	M	Yes	Yes	A, MRI
8	41	F	Yes	No	CT, A, MRI
9	41	F	Yes	Yes	CT, MRI
10	68	M	Yes	Yes	MRI
11	43	M	Yes	Yes	A, MRI
12	37	F	Yes	Yes	MRI
13	49	M	Yes	No	MRI
14	53	F	No	Yes	CT
15	69	M	No	No	CT, A
16	38	F	No	Yes	A
17	40	M	Yes	Yes	MRI

F: female, M: male, CT: computed tomography, A: angiography
MRI: magnetic resonance imaging.

(Tübingen, Germany), using 5.0 and 7.5 MHz transducers.
All patients were examined by the same, experienced sonographer (JS). Patients were placed in a supine position with the neck in retroflexion. B-mode characteristics as well as Doppler flow measurements were recorded.

Results

Carotid body tumors

Of 14 previously diagnosed carotid body tumors 13 were diagnosed with color Doppler imaging. One 1-cm hypervascular tumor in the carotid bifurcation, diagnosed as being a carotid body tumor by magnetic resonance imaging, was not depicted with color Doppler imaging. The absence of splaying of the carotid bifurcation and the close proximity of a vagal body tumor medial to the internal carotid artery may have caused this false negative result.

Vagal body tumors

Of 11 vagal body tumors only 6 were depicted by color Doppler sonography (Table 2-2). Three of these vagal body tumors caused splaying of the carotid bifurcation and appeared as large carotid body tumors. Magnetic resonance imaging and computed tomography showed that in these cases the splaying was more likely

TABLE 2-2 Carotid Body Tumors

Patient	Side	Palpable	Size (mm)	Flow*
1	R	Yes	30 x 30 x 30	++
	L	Yes	10 x 10 x 10	+
2	L	Yes	20 x 20 x 30	++
4	R	No	05 x 05 x 10	++
5	L	Yes	15 x 15 x 25	++
6	R	Yes	10 x 15 x 15	++
8	L	Yes	20 x 20 x 35	++
9	R	Yes	15 x 15 x 15	ND
10	R	Yes	20 x 20 x 20	+
	L	Yes	50 x 70 x 60	++
12	R	Yes	20 x 20 x 20	-
	L	No	05 x 05 x05	++
13	L	Yes	20 x 20 x 30	++
17	R	Yes	15 x 15 x 20	++

R=right, L=left, ND=not depicted
Vascularity: -=none, +=hyper, ++=abundant

TABLE 2-3 Vagal Body Tumors

Patient	Side	Palpable	Size (mm)	Flow*
3	L	No	20 x 20 x 40	++
	R	Yes	30 x 40 x 60	++
4	R	No	15 x 15 x 20	ND
7	L	No	10 x 10 x 15	ND
9	R	Yes	20 x 20 x 25	++
11	R	No	10 x 10 x 15	ND
13	L	No	30 x 25 x 30	ND
14	R	No	20 x 20 x 20	++
15	L	Yes	20 x 30 x 60	++
16	L	Yes	15 x 15 x15	++
17	R	No	10 x 10 x 10	ND

R=right, L=left, ND=not depicted
Vascularity: -=none, +=hyper, ++=abundant

caused by the caudal extension of a vagal body tumor. The demonstration of the remaining 5 vagal body tumors was impaired because of acoustic shadowing from the skull base. Vagal body tumors originating from the nodose ganglion, could only be depicted when large enough to cause a palpable neck mass.

B-mode characteristics

On B-mode sonography, both carotid body tumors and vagal body tumors presented as well-defined solid tumors with low echogenicity and no evidence of a capsule. Smaller tumors were round, whereas larger tumors were ovoid. The smallest diameter was 0.5 cm, the largest was 7 cm. Splaying of the carotid bifurcation was observed with all carotid body tumors larger then 1 cm and 3 vagal body tumors (Figure 2-2). Three tumors grew around the carotids, and 2 of them caused slight compression; none of these tumors had invaded the vessel wall (Figure 2-3). The best results in determining the location of the tumors were obtained with the 5.0-mHZ transducer, whereas the 7.5 MHz transducer allowed more detailed examination.

Doppler flow measurements

With color Doppler imaging, increased vascularity compared with the surrounding was demonstrated in 18 out of 19 tumors. To demonstrate hypervascularity, only 2 tumors required adjustment of the Doppler gain, which was set for the carotid

arteries. In these cases, the Doppler signal tended to bleed out of the natural boundaries of the arteries making appreciation of the tumor difficult.
In 16 cases, abundant flow was seen throughout the entire tumor, mostly in small vessels that could not be visualized with B-mode imaging. The larger vessels showed blood flow velocities comparable to those of the internal carotid artery or even higher. In all cases spectral analysis showed a low-resistance flow pattern.
The diastolic velocity decrease in larger tumors was less than that of the internal carotid artery, and sometimes a high-flow low pulsatility state was seen, as is seen with shunts or arteriovenous fistulas (Figure 2-4). One carotid body tumor showed peripheral hypervascularity with central hypovascularity. This tumor had been embolized 10 years previously. The tumor that showed no hypervascularity by magnetic resonance imaging or color Doppler imaging, was probably a rest of a carotid body tumor extirpated 4 years earlier.

Discussion

The present study shows the color Doppler imaging characteristics in a relatively large series of paragangliomas, including vagal body tumors. Although none of the tumors was confirmed histologically, all patients had multiple hypervascular tumors, 14 patients had a family history of paragangliomas, and 13 patients had had a paraganglioma resected in the past.
Our results confirm earlier reports regarding the sonographic characteristics of paragangliomas.[47;169;195] Although we agree with Derchi et al.[47] that accurately locating a single or bilateral tumor within the carotid bifurcation may lead to the diagnosis of carotid body tumors, we feel that the use of Doppler device, especially color Doppler imaging may contribute to the diagnosis of paragangliomas in several other ways. First, color Doppler imaging can demonstrate hypervascularity with a low-resistance flow pattern within a hypoechoic tumor; this enables discrimination of paragangliomas from lateral neck cysts, Schwannomas, carotid pseudo-aneurysms and most lymph node metastases.[148] Second, examination of vascular structures in the neck is facilitated by color Doppler imaging mostly because of the continuous display of Doppler signal in the B-mode image. This may increase the sensitivity of the examination because small tumors that do not cause the carotids to splay, can be discovered because of their striking hypervascularity. Third, because vagal body tumors do not have a characteristic location, as carotid body tumors do, the demonstration of blood flow is of great importance. However, as we experienced, vagal body tumors, may cause the carotid arteries to splay, and so can be misinterpreted as carotid body tumors. In addition vagal body tumors can be imaged by sonography only if they give rise to a palpable neck mass, and therefore are either large tumors extending downwards from the nodose ganglion, or are rare tumors that occur in the middle and lower part of the vagus nerve. Thus, a negative result on color Doppler imaging does not exclude the presence of a vagal body tumor near the skull base.

FIGURE 2-2A
Longitudinal sonogram demonstrating splaying of the carotid bifurcation by a carotid body tumor. The tumor is ovoid, hypoechoic and hypervascular.

FIGURE 2-2B
Normal bifurcation for comparison.
The blue signal is caused by turbulence at the bifurcation.

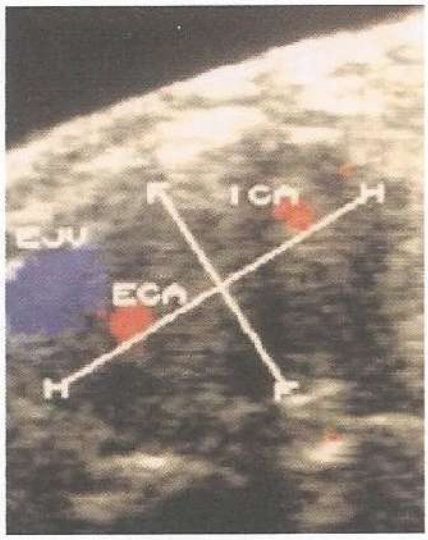

FIGURE 2-3
Transverse sonogram showing a large carotid body tumor (CBT) surrounding the internal carotid artery (ICA) and external carotid artery (ECA). EJV:external jugular vein.

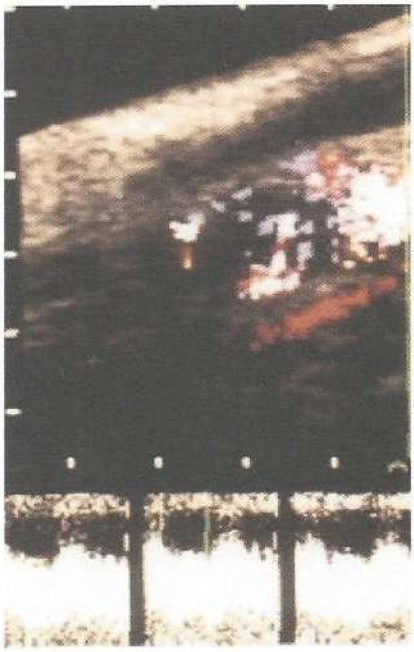

FIGURE 2-4
Longitudinal duplex color scan of carotid body tumor. Spectral analysis of flow at the base of the tumor shows high flow velocities with little systolic-diastolic variation, indicating shunt-like flow.

Because paragangliomas are frequently multicentric, screening of the skull base region, where most vagal body tumors and tumors of the glomus jugulare arise is obligatory in these patients. Color Doppler imaging is restricted to the region of the neck and hence will not replace the more elaborate and expensive examinations by computed tomography and magnetic resonance imaging. Sonography, however, may be the initial diagnostic procedure in the work-up of ambiguous neck masses.[11] Color Doppler imaging can provide valuable information about tumor blood flow and the tumor's relation to the cervical vessels and therefore increases the diagnostic possibilities of sonography. Larger prospective studies color Doppler imaging of both hypervascular and nonhypervascular cervical tumors are needed to evaluate the diagnostic value of this technique.

Chapter 3

Growth Rate of Head and Neck Paragangliomas

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Introduction

The natural course of a disease is of foremost importance when considering treatment strategies. Head and neck paragangliomas (also commonly referred to as glomus tumors or chemodectoma), arise from extra-adrenal paraganglions at various locations in the proximity of the vagal nerve and generally have an indolent growth pattern (Figure 3-1).

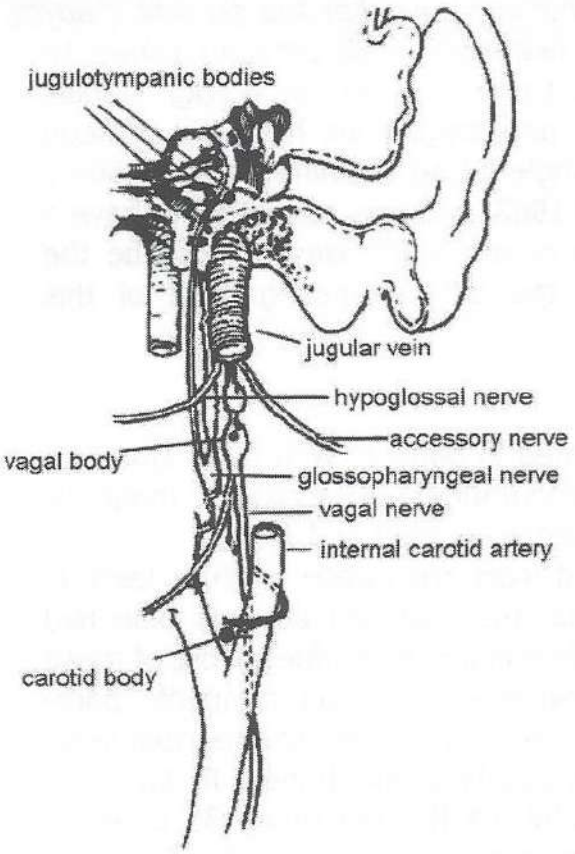

FIGURE 3-1
The extra-adrenal paraganglion system. Schematic drawing of the paraganglion system in the head and neck and its relation to the carotid arteries and lower cranial nerves. Adapted from Zak and Lawson.[217]

Progressive disease may lead to cranial nerve paralysis and eventually to compression of the brainstem. In rare cases patients die of diffuse metastatic spread of the tumor. Although radiotherapy is sometimes advocated most patients are treated surgically. The results of surgical intervention have greatly improved in the past decades. Mortality and the incidence of serious complications like cerebro-vascular accidents have become low; tumors that were considered inoperable are now technically accessible. Nevertheless, in many cases surgery of paragangliomas is complicated by cranial nerve impairment. Surgery of vagal body tumors invariably leads to loss of the vagal nerve because the tumor arises at the vagal ganglions and encases the nerve. If vagal body tumors invade the base of skull (usually through the jugular foramen) the operative morbidity increases. Of all head and neck para-gangliomas the natural course of jugulo-tympanic tumors causes most morbidity. However, even in these cases surgery leads to a twofold increase of cranial nerve palsy. Surgery of carotid body tumors causes cranial nerve impairment in >10% of the cases. Therefore the risk of serious complications as a result of treatment

remains an important factor in the treatment decision making for individual patients and must be weighed against the natural course of these tumors. We previously published a clinical study that showed little benefit of surgery for paragangliomas occurring at the base of skull. In this series all patients operated or not had a life expectancy that was close to normal, whereas the group of patients who underwent surgery suffered the most complications. For this reason we have become more cautious when performing surgery for vagal and jugulotympanic paragangliomas and we have acquired a large amount of follow-up imaging-data. For the present study we used the radiological data of 48 untreated head and neck paragangliomas to calculate tumor doubling times (T_d) of these rare tumors. To our knowledge studies addressing the growth rate of head and neck paragangliomas have never been published (Medline search 1967-1999). Only a single intrapulmonary paraganglioma was assessed radiographically between 1946 and 1962, and was calculated to have a tumor doubling time of 5.4 year. The objective of our study was to describe the growth pattern of paragangliomas to improve the difficult management of this tumor.

Patients and Methods

Tumor size and tumor doubling time were estimated in patients with head and neck paragangliomas who, underwent computed tomography (CT scan) or magnetic resonance imaging (MRI) in the study institution between 1986 and 1996,

Of a total of 53 patients, 26 who were examined more than once (with at least 1-year interval) were included in the study. Among the excluded patients nine had paragangliomas that were extirpated after a single imaging procedure. Most of these patients had small carotid body tumors (6 patients) or small tympanic paragangliomas (2 patients). One patient underwent surgery for intracranial extension and compression of the brainstem by a large jugulo-tympanic tumor. In 13 cases follow-up was not performed in the study hospital. Of the remaining 31 cases, 5 were excluded because tumor size could not be measured satisfactorily; 1 jugulotympanic paraganglioma was surrounded by infection and 4 CT-scans were of insufficient quality to estimate the tumor boundaries. Finally 26 patients, with 48 paragangliomas were included in the study. The number of hereditary cases of head and neck paragangliomas who attend the study institution explains the unusually high percentage of patients with multiple tumors (79%).[185]

Computed tomography was performed with a Tomoscan 350 (Philips, Best, The Netherlands). Three millimeter slices were made with and without intravenous contrast enhancement and printed in bone and soft tissue settings. MR imaging was performed on a 1.5T system (Philips, Best, The Netherlands) using a quadrature head coil. Both plain and contrast enhanced T_1- weighted and T_2- weighted images were used for optimal delineation of the tumor. The slice thickness of the MR sequences varied from 7 mm with a 0.7 mm interslice gap for the earlier examinations to 5 mm with a 0.5 interslice gap for the later studies. All measurements were performed on hardcopies. The largest diameter in antero-posterior and medio-lateral direction was measured with a millimeter caliper. The

cranio-caudal size was estimated to be the product of slice thickness (including the interslice gap) and the number of slices in which the tumor could be identified.

Tumors were considered to have a ellipsoid shape and the following equation was used to estimate the tumor volume:

$$V = \frac{4}{3}\pi\left(\tfrac{1}{2}A \times \tfrac{1}{2}B \times \tfrac{1}{2}C\right)$$

in which V = volume, A = the largest dimension in antero-posterior direction, B= th e largest dimension in medio-lateral direction and C =the largest dimension in cranio-caudal direction.
According to a study by Lundin et al. (1992) the measurement error was expected to be at least 15%.[129] To distinguish growing paragangliomas from stationary tumors we considered a volume increase of 20% a minimum.
The tumor doubling time was estimated using the following equation:

$$T_d = (T_2 - T_1) \times \left(\frac{\log 2}{\log V_2 - \log V_1} \right)$$

in which T_d = tumor doubling time, T_2 = second imaging time, T_1 = first imaging time, V_2=volume at T_2 and V_1 = volume at T_1.
The data regarding tumor doubling time were supplemented with the estimation of the increase of dimension per annum. Because the cranio-caudal diameter was measured less accurately, growth-rates were calculated from the largest increase in diameter in the axial plane.

Statistics
Tumor volume and growth parameters were not distributed normally, therefore median values rather then means are given, and distribution free methods were used for statistical analysis (Spearman's rank correlation coefficient (ρ) for correlation and Wilcoxon rank sum test for comparison of sample distribution).

Results

The 26 patients studied had a total of 48 paragangliomas: 20 of the carotid body, 17 of the vagal body and 11 of the jugulo-tympanic bodies. The follow up period was 1 to 8 years with a mean of 4.2 year. The initial tumor volume varied between 0.07-58.39 cm^3 (median 2.46 cm^3). The distribution of tumor doubling time is illustrated in Figure 3-2.

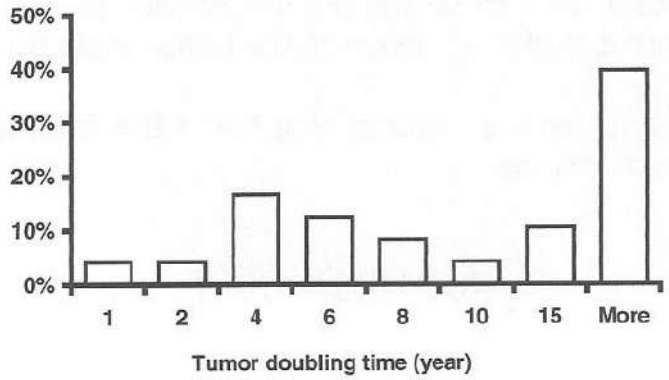

FIGURE 3-2
Tumor doubling time of paragangliomas (n=48). Histogram showing the frequency distribution of tumor doubling times. The majority of the paragangliomas had a tumor doubling time >10 years. The median tumor doubling time in the growing group was 4.2 years.

The median increase in diameter was 0.83 mm/year and that for tumor doubling time 10.15 years for the complete group. In ten cases the volume was unchanged, whereas in two tumors a slight decrease in volume was estimated. A volume increase of >20% was noted in 29 paragangliomas (60%); these tumors were considered to be growing. The median increase in diameter in this sub-group of growing tumors, was 1 mm/year (0.3-5 mm/year) and the median tumor doubling time (T_d) was 4.2 years (range 0.6-21.5 years).
Comparison between different tumor locations showed that jugulo-tympanic paragangliomas tended to be smaller and less progressive than paragangliomas developing at other locations (P=0.08) (Table 3-1). No significant difference between the growth of hereditary tumors (n=39, median T_d =8.7 years) and sporadic cases (n=10,median T_d =18.9 years) was observed (P=0.328).
Approximately 67% (n=32) of the paragangliomas gave rise to signs or symptoms. The symptoms were generally mild. None of the paragangliomas caused cranial nerve palsy, although two patients had pre-existent loss of function after surgery for jugulo-tympanic paragangliomas at the same side. Hearing loss and tinnitus were present in all but two patients with jugulo-tympanic paragangliomas.
Symptomless paragangliomas were statistically smaller than those causing symptoms ($p<0.001$), but no significant difference in growth rate was present. In 38 cases (79%) the symptoms did not change during follow up even if the tumor volume increased by > 20%. In the group without growth one patient developed perceptive hearing loss and in another case a previously not noted swelling was palpated at the time of follow up.
Correlation between initial tumor volume and T_d is shown in Figure 3-3.
Because a large proportion of the very small tumors did not grow this correlation was weak for the complete group ($\rho=0.23$) (Figure 3-4).
If growing paragangliomas were regarded separately the correlation was strong ($\rho=0.66$, $p=0.002$) indicating a lower growth rate in larger tumors.

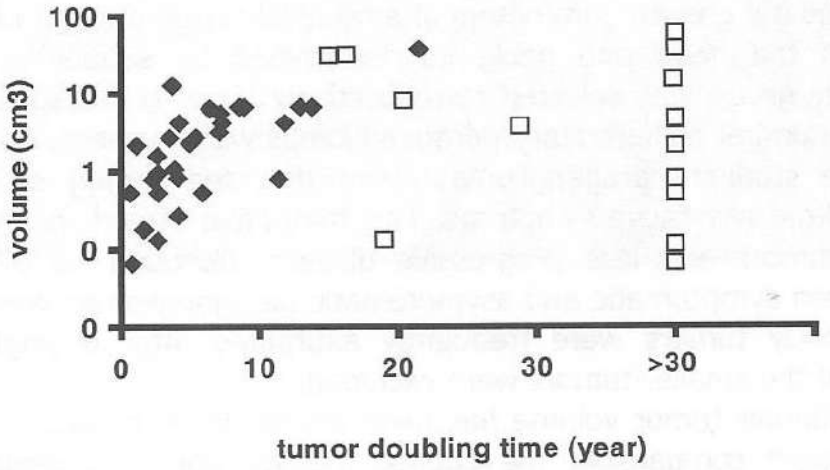

FIGURE 3-3
Correlation between tumor doubling time and volume of paragangliomas. Scatterplot with different symbols for growing paragangliomas (solid diamonds) and stationary paragangliomas (open squares).

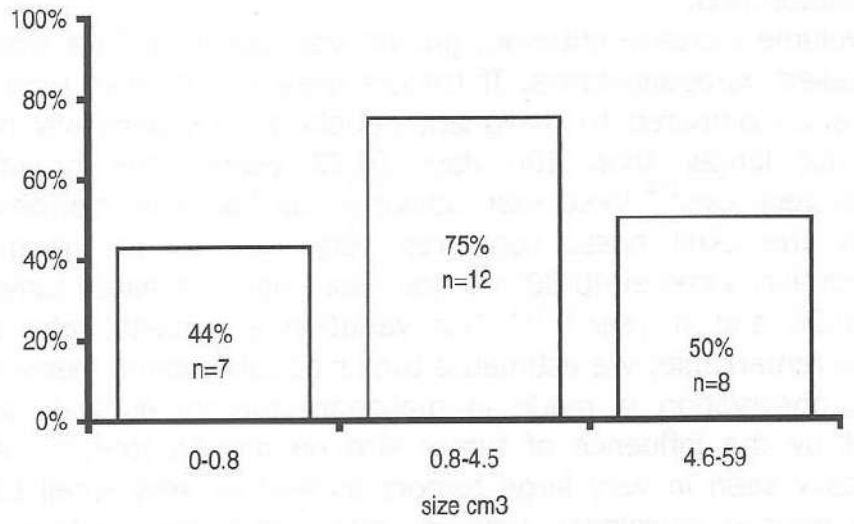

FIGURE 3-4
Percentage of growing paragangliomas. The total tumor group was divided into three groups of approximately equal number. Tumors with the same size were kept in the same group. The figure shows that growth was observed mainly in tumors of intermediate size. Differences between the three groups were not found to be statistically significant.

Discussion

This study describes the growth parameters of a relatively large number of untreated paragangliomas in the head and neck, as determined by sequential radiologic imaging. The study group was selected retrospectively. Due to our specific patient population a high number of hereditary paragangliomas were present. Consequently a number of the studied paragangliomas were detected during screening for multicentricity and did not cause symptoms. This may have biased the study group towards smaller tumors and less progressive disease, although no difference in growth rate between symptomatic and asymptomatic paragangliomas was observed. Because carotid body tumors were frequently extirpated after a single imaging procedure, some of the smaller tumors were excluded.

The method to estimate tumor volume has been discussed by others.[114;129;153;216] If used for intra patient comparison, the applied method has an acceptable error, calculated to be between 13% and 18% in pituitary tumors.[129] However, one must recognize that the measured volumes are estimates and changes in volume may be more accurately reflected than absolute tumor volumes. We considered a 20% increase in tumor volume as a minimum for proof of tumor growth. The fact that the maximum *decrease* in tumor volume measured in this series was 10.5%, an observation likely due to measurement errors, illustrates the belief that such a margin must be established.

Using the 20% volume increase criterion, growth was observed in a small majority (60%) of the studied paragangliomas. If tumors grew the median tumor doubling time was 4.2 years. Compared to malignant neoplasm that generally have tumor doubling times not longer than 100 days (0.27 years), the growth rate of paragangliomas is very low.[194] Vestibular Schwannoma (acoustic neuroma) another benign tumor at the skull base, compares very well to paraganglioma with radiological progression noted in 40-80% of the cases and calculated tumor doubling times between 0.56 and 8 year.[35;114] The variation in growth rate of growing paragangliomas is remarkable; we estimated tumor doubling times between 0.6 and 21.5 years. This observation is made in malignant tumors as well and can be explained in part by the influence of tumor size on growth rate.[2;194] Absence of growth was typically seen in very large tumors as well as very small tumors. Our observation of a positive correlation between size and tumor doubling time is in accordance with a model of retarded growth. Because most paragangliomas grow expansively, the reason for this growth retardation might be the resistance of surrounding tissue. This would also explain the smaller size and longer doubling time of jugulo-tympanic paragangliomas since these tumors are confined to a space largely surrounded by the petrous bone. However, the small size of jugulo-tympanic tumors can also be explained by early detection due to the early onset of symptoms (hearing loss and tinnitus). Poor vascularization of large tumors is another frequently suggested reason for growth retardation. However, paragangliomas are extremely well vascularized and central hypovascularity or necrosis is seldom observed. To our knowledge DNA-ploidy studies and immunohistochemistry of paragangliomas to date have shown that these tumors are heterogeneous in DNA-ploidy and expression of

different proliferation markers.[74;76;141] This heterogeneity could reflect differences in growth rate, but this has yet to be investigated.
The observation that only 44% of the very small paragangliomas was growing, compared to 75% of the tumors of intermediate size, is remarkable. It is possible that the clonal expansion in head and neck paragangliomas is preceded by a hyperplastic growth phase that, in combination with growth retardation in large paragangliomas, leads to a biphasic growth curve.
In vestibular schwannomas (acoustic neuromas), that clinically behave very much like paragangliomas and present similar surgical problems, radiological studies have been helpful to evaluate the natural course of these tumors. (Reviewed by Charabi 1997)[35] Several authors have suggested a wait and see policy for vestibular schwannomas, depending on age, the hearing in the other ear and bilateral tumors.[46;124;153;198;203;209] The natural course of the paragangliomas described in this study justifies a similar approach for these tumors. Of course one must be aware of the fact that the estimation of tumor doubling time can predict only partially the clinical course of the disease. Cranial nerve impairment (being the foremost complication of this tumor) depends also on the direction of growth and the local behavior of the paraganglioma.[56] Nonetheless, the absence of growth in 40% of the tumors and the doubling time of more than 3 year in 81% of the cases, suggests that a 'wait and scan' policy is justified in case the patient or the surgeon hesitates whether should be performed.
The management of hereditary cases of paraganglioma is a special problem because they frequently develop bilateral tumors and are therefore at risk for extremely debilitating bilateral cranial nerve palsies. The advent of genetic counseling in paraganglioma-families and subsequent MRI-screening of carriers, has led to the detection of pre-symptomatic paragangliomas and introduced new problems.[69;160] Small pre-symptomatically detected tumors might be extirpated with a minimum of morbidity, but -as shown in the current study- a large proportion of these paragangliomas is not progressive (at least for many years), and will not necessarily cause symptoms in the future. Radiologically proven progression can make the decision to treat easier, and makes surgical complications more acceptable. In our opinion a 'wait and scan' policy must be one the treatment strategies that are considered in each case of this intriguing tumor.

Chapter 4

The Risk of Head and Neck Paragangliomas in SDHD Mutation Carriers

The Risk of Head and Neck Paragangliomas in SDHD Mutation Carriers
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AJMG, accepted for publication

Chapter 4

The Risk of Head and Neck Paraganglioma in SDHD Mutation Carriers

Introduction

Head and neck (HN-) paragangliomas are rare, predominantly benign tumors that arise from extra-adrenal paraganglion tissue associated with the parasympathetic nervous system. In decreasing order of frequency the carotid body in the carotid bifurcation, the jugulo-tympanic bodies at the jugular bulb and tympanic nerve and the vagal bodies at the ganglions of the vagal nerve are affected. The tumor is most frequently diagnosed in adults and characteristically progresses slowly. Local extension may lead to cranial nerve impairment and eventually compression of the brain stem. Between 10% and 50% of the published cases of HN-paragangliomas are hereditary.[138;143] Although the natural course of hereditary cases does not seem to be different from sporadic paragangliomas, patients with inherited disease are more likely to develop multiple paragangliomas.[163]

The inheritance pattern of HN-paragangliomas is peculiar in that the children of female patients are never affected. Individuals are at risk only when they receive the mutant allele from their father, regardless of his clinical status[143]. This has been explained by genomic imprinting, a reversible parent-of-origin-dependent gene-inactivation process.[58] Linkage analysis has yielded two loci involved in HN-paragangliomas of which PGL1 at 11q23 is the most common.[17;91;184] Thus far PGL2 at 11q13 has been associated with HN-paragangliomas in a single family.[133] Recently PGL1 has been identified as the SDHD gene that encodes for a mitochondrial respiratory chain protein (succinate-ubiquinone-oxidoreductase-subunit-D).[18]

Because paragangliomas can cause debilitating symptoms and family members may have been treated surgically with the potential for serious complications, knowledge of being at risk for this disease can cause great distress. Proper genetic counseling of presymptomatic carriers requires estimates of the risks conferred by SDHD mutations for developing HN-paragangliomas. Only one previous study addressed this issue, reporting the penetrance to be complete by the age of 45.[10] However, this study was performed prior to the establishment of the parent-of-origin effect and prior to the identification of PGL1 and PGL2.[133] ID:785} In addition, we have found that screening with magnetic resonance imaging (MRI) for HN-paragangliomas can reveal symptomless tumors in older patients.[69] It is therefore possible that the penetrance of the gene is not complete, and may differ substantially depending on the clinical ascertainment. Here we show that SDHD has reduced penetrance by age 70 when only clinical symptoms are taken into account, but is completely penetrant at this age when MRI screening results are added. The presented data can be used to tailor the screening program of proven gene carriers.

Patients and Methods

Clinical status

The disease status of 195 relatives belonging to a 7-generation family with HN-paragangliomas (FGT189) was established between 1990 and 1997. The pedigree of this family has been published before.[69;160;184]

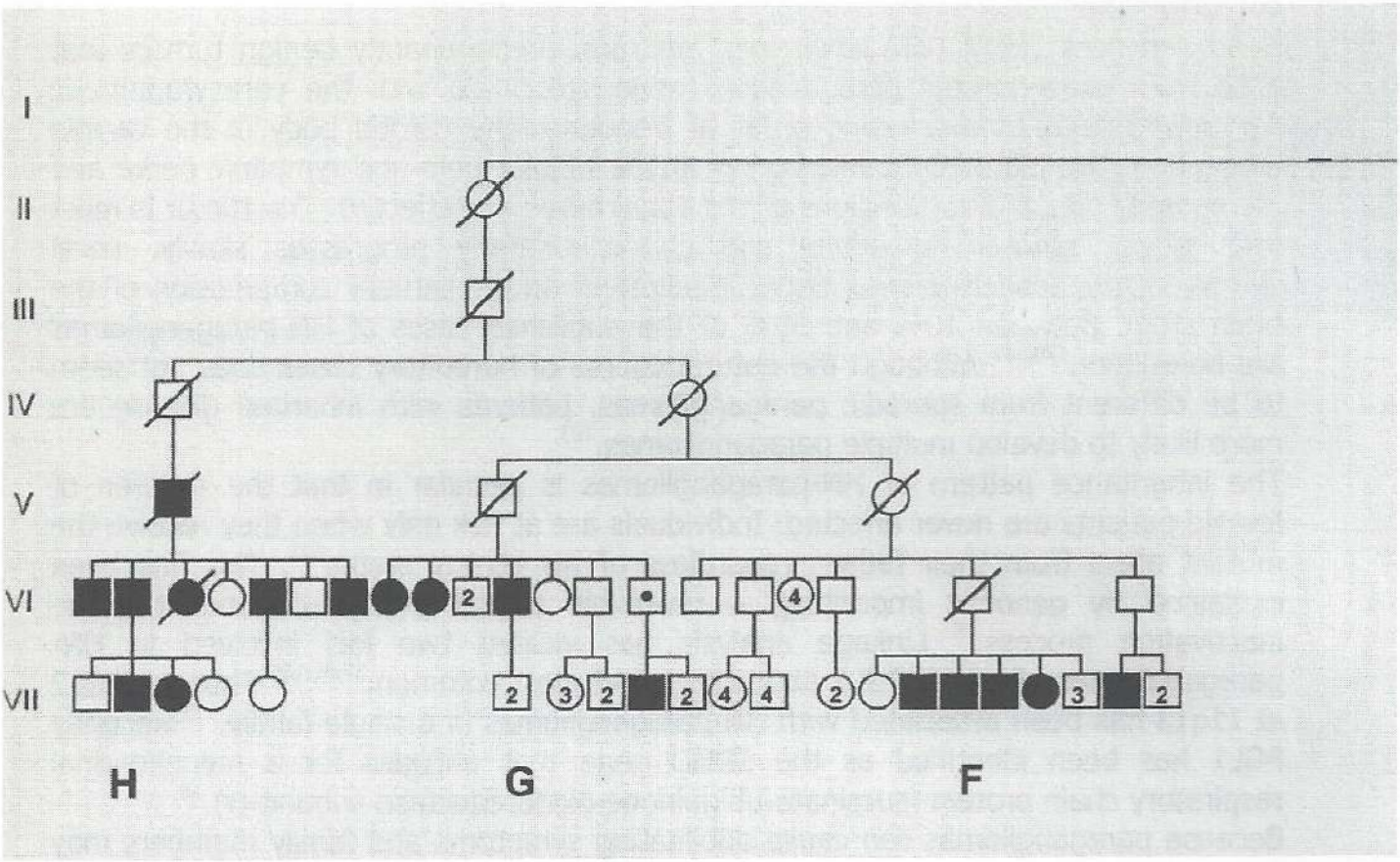
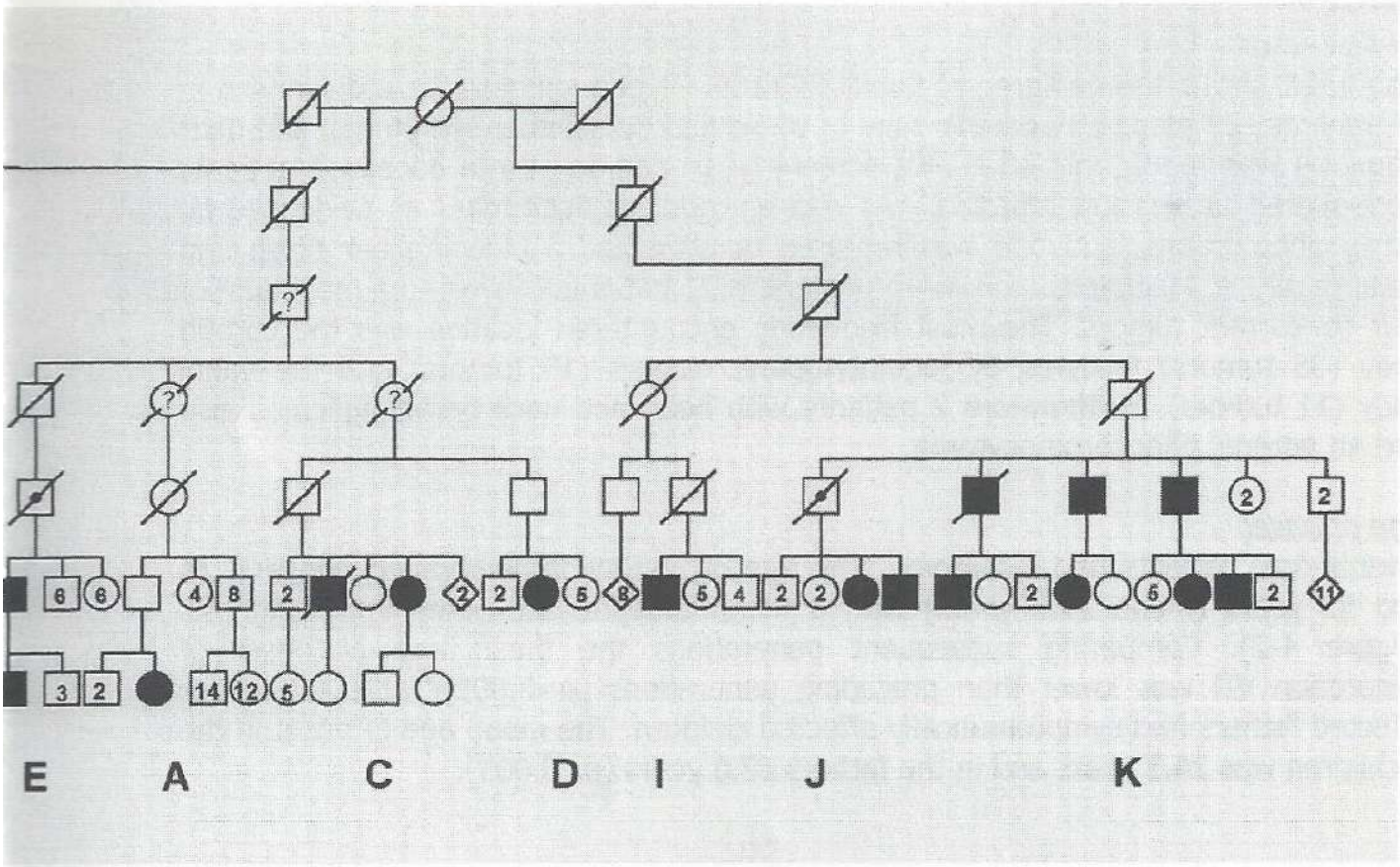


FIGURE 4-1 Family FGT189
Square: male; circle: female; diamond: multiple sibs of both sexes; dotted symbol: obligate carrier of paternally inherited mutation; question mark in symbol: possibly affected; number in symbol: number of sibs.

Family members underwent magnetic resonance imaging (MRI) if they showed signs or symptoms of paragangliomas. Non-symptomatic paternal gene carriers that were identified during genetic counseling were offered MRI screening as well. Moreover we could use the data acquired in a previous research protocol in which 83 members of this family were examined with MRI, regardless of their disease status.[69].
In deceased family members the medical history was examined to evaluate the likely clinical status.

Genetic status
Genomic DNA from family members who had donated a blood sample was genotyped at a number of polymorphic DNA-markers. These markers defined an approximately 10-cM interval on 11q22.3-q23.1 that contained the SDHD gene.[19;185] All patients in family FGT189 shared a haplotype defined by these markers, and this haplotype was recently shown to harbor a missense mutation (g.7882 T>C; Asp92Tyr) in SDHD.[18]



Age of onset

The age of onset of HN-paragangliomas is difficult to establish because the initial symptoms are mild and patients may take years to come under clinical attention. We therefore made a difference between *age of onset* i.e. the age at which the patient retrospectively experienced symptoms of a head and neck paraganglioma, and *age of diagnosis* i.e. the age at which the diagnosis was confirmed radiologically.
Baysal et al. described a lower age of onset in children than in their affected fathers, we therefore compared the age of onset in father-child pairs and the age of onset according to generation.[17]

Penetrance

Given the typical inheritance pattern of HN-paragangliomas, children of female carriers were considered not to be at risk. To estimate the penetrance we only used the data of generations VI and VII. Although some patients were ascertained in the older generations they were not included because insufficient data was available from their sibs to identify non-penetrant carriers. All calculations were made with and without inclusion of the symptomless patients. First we used the method described by van Baars at al. and considered 50% of the children of affected fathers as well as sibs of affected individuals to be at risk.[9] Subsequently we combined the genetic and clinical data to estimate the penetrance. The penetrance was expressed as a Kaplan-Meier curve, representing the probability of a gene carrier to be disease free at a given age.

Results

Clinical status

Figure 4-1 shows the pedigree of family FGT189. Paragangliomas were diagnosed in 33 patients (22 men, 11 women). Nine of these had no signs or symptoms, and their diagnosis was made only after MRI screening. In addition three deceased patients were scored as possibly affected: two had an indolent neck mass as evidenced by family photographs, and one was reported to have had a bleeding ear polyp and facial paralysis. Multiple tumors were present in 21 patients (64%), to a maximum of four concurrent tumors. The most frequently encountered location was the carotid body (35 tumors) followed by jugulotympanic tumors (15 tumors) and the vagal body (11 tumors). Furthermore 2 patients with head and neck paragangliomas also had an adrenal pheochromocytoma.

Age of onset

Twenty-four patients had symptoms. The age of first symptoms varied between 14 and 53 years (mean 29.3 years) with a mean delay of 3.2 years until diagnosis (Figure 4-2). Comparing subsequent generations the mean age of onset of generation VII was lower than preceding generations (p=0.002)(Table 4-1). Four affected fathers had symptomatically affected children. The mean age of onset in the 7 children was 24.3 years and in the fathers 37.0 years (p=0.003).

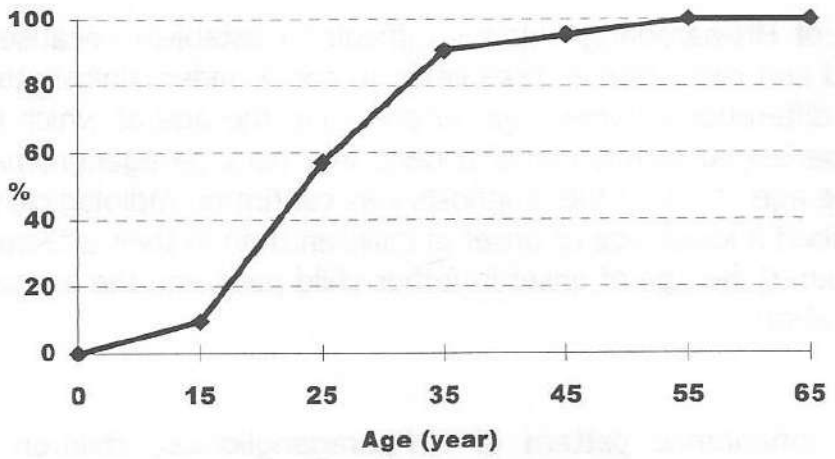


FIGURE 4-2. Age of onset
Cumulative recording of the age at which the first symptoms of a head and neck paraganglioma appeared in 24 family members.

TABLE 4.1 Age of Onset According to Generation

Generation	Symptomatic cases	Mean age of onset
V	3	30.7
VI	13	30.5
VII	10	20.4[1]
total	26	29.3

[1]P=0.002 (student-T test)

TABLE 4-2 Penetrance in Generation VI and VII

Paternal disease haplotype			Symptomatic Paragangliomas			MRI diagnosed Paragangliomas		
Total	Male	Female	Total	Male	Female	Total	Male	Female
44	27 (61%)	17 (39%)	23 (52%)	14	9	29 (66%)	18	11

Genetic status

Haplotype analysis of 169 family members identified 59 carriers of the disease gene; 11 received the gene from their mother and were considered not to be at risk for paragangliomas. One case carried a recombinant haplotype, and we were unable to unambiguously determine whether this individual carried the mutation. The result of mutation analysis in this case, performed recently after identification of the SDHD-gene mutation, is not known to the patient and can not be disclosed here for confidentiality reasons. MR-imaging of this male failed to reveal any paraganglioma.

Penetrance

Penetrance of the disease was dependent on the sex of the transmitting parent. No paragangliomas were found in the offspring of female carriers. The pedigree shows that even some of the older family members who received the paternal disease gene remained clinically unaffected. Three male obligate carriers in generation V and VI, who inherited the gene from their father, did not develop symptoms of paragangliomas. Two of them died at ages 80 and 72, respectively. The third carrier is alive and clinically free of symptoms at last contact. He refused MRI.

We identified 24 male carriers in generations V and VI on the basis that they were either affected themselves or had affected offspring. Their children (n=81) have a 50% chance of inheriting the mutation. Twenty-nine of the children at risk were affected, 23 of whom had symptoms. Clinically the penetrance was therefore 57%.

Using the genetic data, 44 paternally derived gene carriers were identified in generation VI and VII (Table 4-2). Of these persons 23 displayed symptoms of paragangliomas accounting for an overall penetrance of 23/44 or 52%. To correct for the age of onset we made an actuarial survival curve representing the chance to

be symptom free as a function of time (Figure 4-3a). Since none of the carriers developed symptoms after the age of 53 the penetrance reached a maximum of 66% at this age.
Of the 21 clinically non-penetrant carriers of the disease gene, 10 were examined with MRI. In 6 cases one or more paragangliomas were diagnosed, raising the overall penetrance to 29/44 or 66%. If these cases were included in the Kaplan Meier curve the penetrance increased to 100% by 70 years of age (Figure 4-3b).

Discussion
Hereditary paragangliomas of the head and neck present a unique tumor syndrome that does not conform to Mendelian rules of inheritance. The penetrance of the disease gene is zero if it is transmitted by a female. This has considerable consequences for the genetic counseling of afflicted families. Children of female carriers can be dismissed from their disease risk, but must be aware that they can transmit the disease gene to their own children. Carriers of a paternally inherited disease gene however, must be informed that they are at risk for head and neck paragangliomas. Although tumors develop later in adult life and seldomly metastasize, they can, in a minority of cases, cause serious symptoms and require extensive surgery. For these patients accurate risk assessment is an important aid in planning clinical decisions. We have here provided these risk estimates and have demonstrated that these risks differ considerably depending on whether or not MRI-screening results are included.
In the presented family not all at risk individuals developed clinical symptoms despite that some of them reached advanced ages. This conflicts with the observations made by van Baars et al. who estimated the penetrance of the disease to be complete at the age of 45. Even more surprising is that this study regarded children of affected females to be at risk, under the assumption of a normal autosomal dominant inheritance pattern. In this way 12 children that retrospectively were not at risk were included in a total of 57 studied siblings. We used the same methodology in a family of comparable size, but excluded children of female carriers, which nevertheless yielded a considerably lower penetrance. Using Kaplan-Meier survival analysis strictly for proven gene carriers with symptoms of head and neck paragangliomas still yielded a penetrance of only 66% by age 53. Only upon inclusion of the magnetic resonance imaging results in non-symptomatic carriers were we able to demonstrate that the gene was fully penetrant at age 70.
Because only two families have thus far been examined regarding this issue, the differences in penetrance are probably not significant. Since pedigrees with a large number of affected individuals are rare occurrences in the general population, the actual penetrance may in fact be lower.[62] Not counting the index case (Weinberg's proband method) provides a simple correction for the ascertainment bias. If applied to the families described by van Baars et al. the overall penetrance would be 59%. In 10 families attributed to the same ancestral mutation as the family described in this paper, it yields an overall penetrance of 44% (data not shown), which is lower than the overall penetrance we calculated in family FGT189 alone.[185] However, the

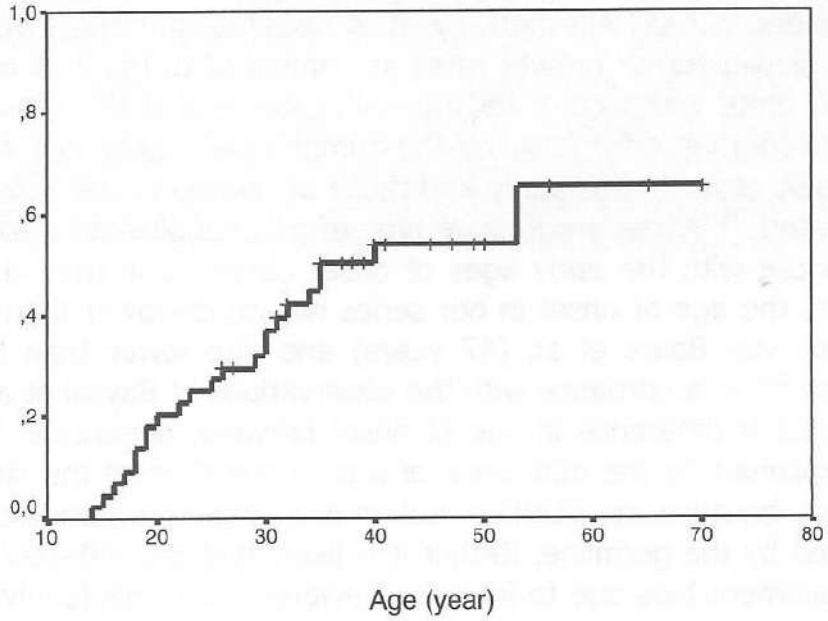


FIGURE 4-3a Penetrance of symptomatic head and neck paragangliomas. Inverted Kaplan-Meier curve indicating the chance to develop symptoms (Y-axis) at a certain age (X-axis) for carriers of a paternally inherited SDHD mutation.

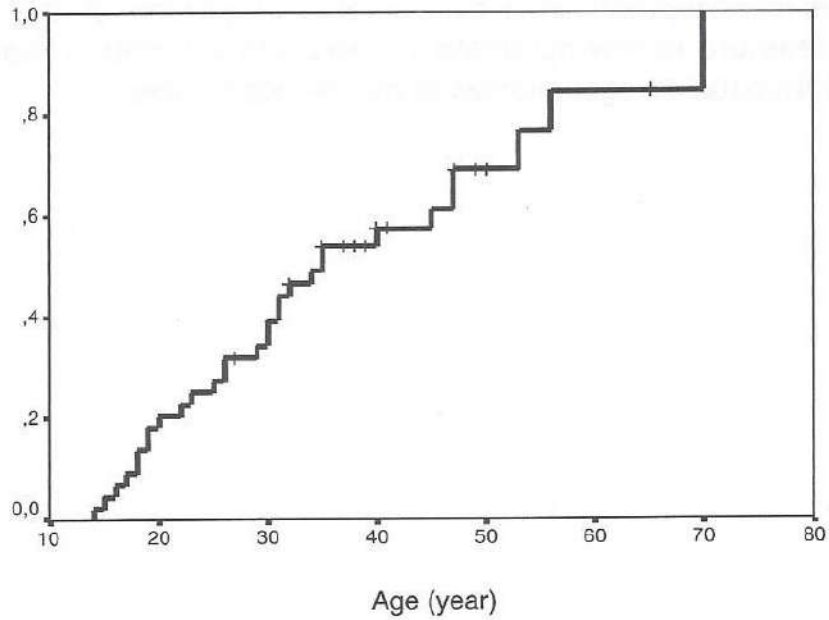


FIGURE 4-3b Penetrance of head and neck paragangliomas including presymptomatic cases. Inverted Kaplan-Meier curve indicating the chance to develop a MRI detectable paraganglioma (Y-axis) at a certain age (X-axis) for carriers of a paternally inherited SDHD mutation.

family described by van Baars was later linked to PGL2 at 11q13, and we can therefore not rule out that the different penetrance estimates reflect a difference in the underlying genetic defect. Alternatively, it is possible that the involved SDHD mutation leads to slower tumor growth rates as compared to PGL2. A recent study on tumor doubling time, which consisted mainly of carriers of the SDHD mutation studied here, showed that only 60% of the tumors were growing. A difference between the sporadic cases in this study and those attributed to the SDHD mutation was not demonstrated.[100] A less progressive type of paraganglioma in SDHD carriers is difficult to reconcile with the early ages of onset observed in this study. With a mean of 29.3 years the age of onset in our series was much lower than that of the family described by van Baars et al. (47 years) and also lower than most series described by others.[163] In accordance with the observations of Baysal et al.[17] we saw a statistically significant difference in age of onset between generation VI and VII. This cannot be explained by the difference of age at the time of the study, nor to genetic anticipation, because the SDHD mutation is a single nucleotide change that is stably transmitted by the germline. Rather it is likely that the difference in age of onset is an ascertainment bias due to increased awareness in this family that led to detection of smaller tumors in children of affected parents. Head and neck paragangliomas are very slow growing and thus detection of a slightly smaller tumor has considerable impact on the age of onset. In this regard it must be stressed that we used the age of first symptoms rather then the age of diagnosis to calculate age of onset and that we excluded cases diagnosed through screening MRI.

We conclude that all carriers of a paternally inherited SDHD mutation may eventually develop head and neck paragangliomas though the slow growth rate of these tumors prevents the onset of symptoms in a considerable proportion of the cases. This knowledge might reassure non-symptomatic carriers and warrants a 'wait and scan' policy for non-symptomatic paragangliomas in middle aged patients.

Chapter 5

Confinement of PGL1 to a 2-cm Interval on 11q22-q23

Published as:
Confinement of PGL, an Imprinted Gene Causing Hereditary Paragangliomas, to a 2-cm Interval on 11q22-q23 and Exclusion of DRD2 and NCAM as Candidate Genes
Schothorst van EM, Jansen JC, Bardoel AFJ, vd Mey AGL, James MJ, Sobol H, Weissenbach J, v Ommen GJ, Cornelisse CJ, Devilee P. Eur J Hum Genet 4:267-273, 1996

Introduction

Paragangliomas of the head and neck region, also known as glomus tumors or chemodectomas, are slow growing, mostly benign tumors of neuroectodermal origin. Their incidence has been estimated to be approximately 1:100,000 and they manifest roughly between the age of 18 and 60.[217]

The familial form of the disease displays an autosomal dominant mode of inheritance.[143;217] However, the tumors only develop in individuals who have inherited the gene paternally, whereas maternal transmission results in non-affected carriers only.[143] This has been interpreted as evidence that the underlying gene defect is subject to 'genomic imprinting', an epigenetic mechanism whereby, in a reversible process, a gamete-specific modification in the parental generation leads to functional differences between maternal and paternal genomes in the offspring.[13;143]

In an earlier study we have mapped the disease gene, termed PGL (OMIM 168000), by linkage analysis in one large Dutch family (FGT01) to chromosome 11q22-q23.[91] The reported meiotic recombinants in the families positioned PGL between the markers STMY and CD3D, separated at a 26 cM genetic distance.[92] More recent estimates fix this distance at 16 cM.[49] Although no statistical evidence was obtained for the involvement of genetic heterogeneity[92], a single family has been shown to link to markers for 11q13, and not to be due to the PGL locus on 11q22-q23.[133;134] This suggests that a second locus may exist.

Because paragangliomas are rare, few families become available for recombinant analyses, and the current size of the gene region of 16 cM is too large to seriously attempt the positional cloning of PGL. We present here the detailed haplotype analysis of this region in a single Dutch multibranch 7-generation family. This allowed the definition of a shared haplotype between all affected descendants, narrowing the candidate gene region to an interval of 2 cM between the markers D11S938/D11S4122 and D11S1885.

Materials and Methods

Family ascertainment

Diagnosis of paraganglioma was based on medical history, physical examination, and/or determination of free urinary catecholamine excretion.[91;143;160] In 94 individuals, magnetic resonance imaging (MRI) of the head and neck region was performed as well.[67] Part of the large family (branches E-H in this study, Figure 5-1) has been presented earlier as FGT01, in which the initial linkage was obtained.[91] Branches A, C and D were previously presented as family FGT09.[92]

DNA isolation and PCR analysis

Blood samples were collected from 190 individuals and genomic DNA was isolated from peripheral blood lymphocytes as described by Miller.[145] PCR conditions to visualize microsatellite polymorphisms were as described.[92;210] All primer sequences for these markers are retrievable online from GDB or Généthon databases, and all oligonucleotides were manufactured by Isogen Inc.

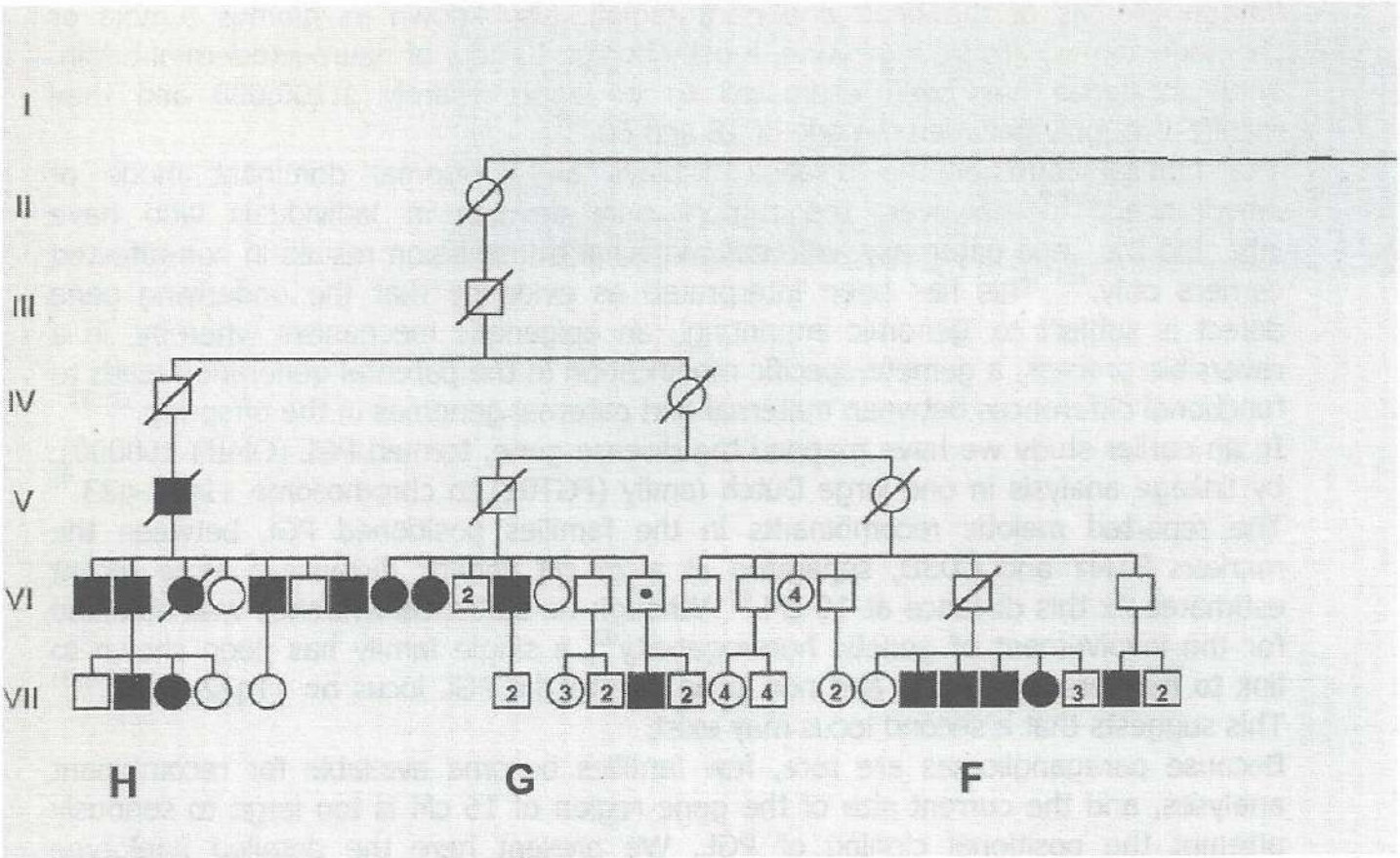
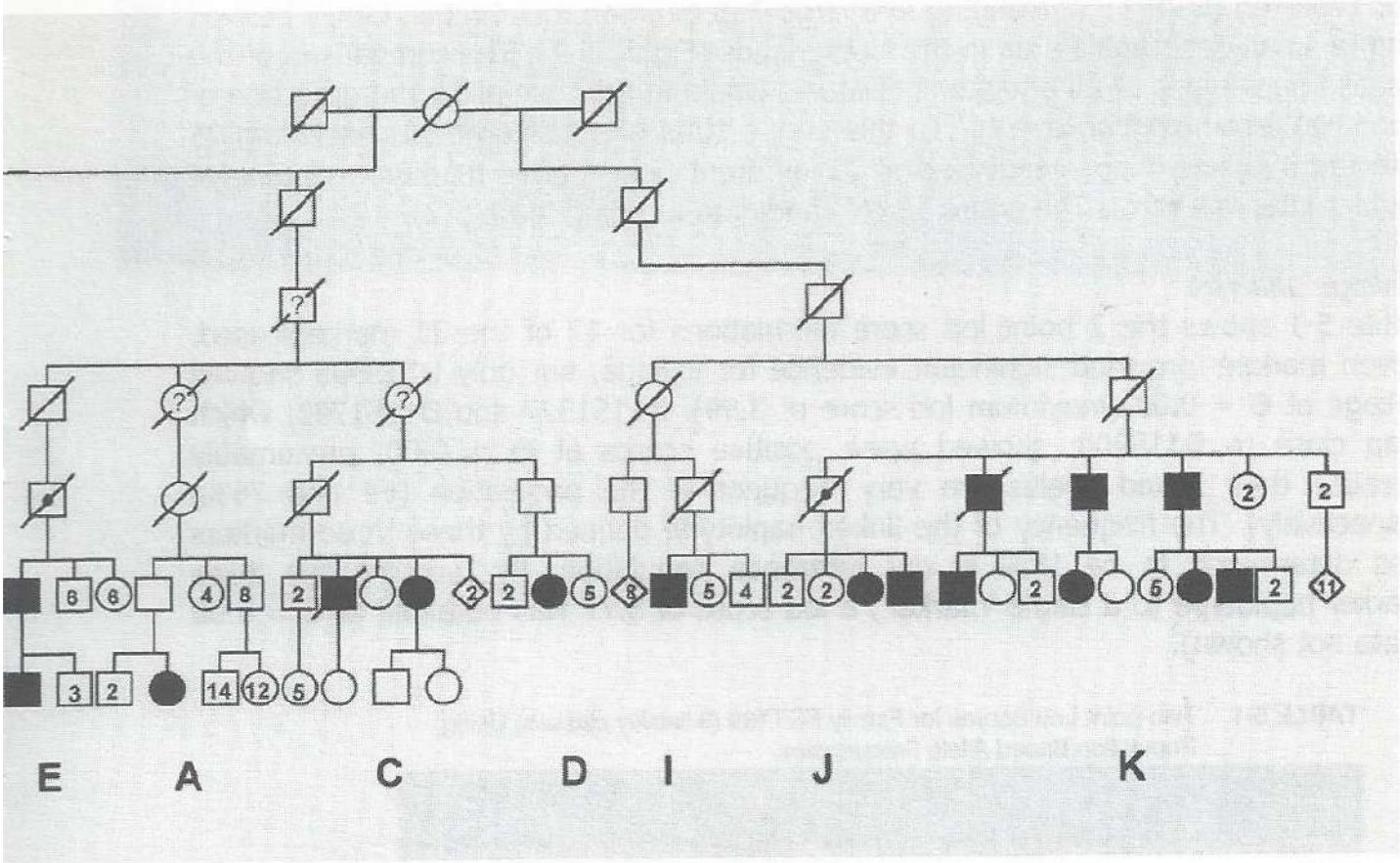


FIGURE 5-1 Family FGT189.
Square: male; circle: female; diamond: multiple sibs of both sexes; dotted symbol: obligate carrier of paternally inherited mutation; question mark in symbol: possibly affected; number in symbol: number of sibs. The letters (A-K) correspond with the branches referred to in the text. The pedigree is updated in 2000, and includes several newly diagnosed patients.

Linkage analysis

Linkage analysis was performed using the LINKAGE program package version 5.1.[118] Briefly, 8 liability classes were defined to account for age of onset and the absence of penetrance in children of female gene carriers.[91;92] The population incidence of glomus tumors has been estimated to be 1:100,000, but we believe this is probably an underestimation. In addition, because the disease shows incomplete penetrance, the number of gene carriers is probably higher than the number of patients. Hence we have used a conservative estimate for the disease gene frequency of 0.001 in order not to inflate lod scores. Allele lengths of the markers were determined using an M13 sequence as reference, and were as expected from GDB. Lod scores were computed using allele frequencies that were determined in 41 unrelated Dutch individuals from the same area in the Netherlands from which the 7 generation family originated. Twenty of these were spouses marrying into this family, the others were spouses marrying into 3 families with familial atypical multiple mole melanoma syndrome.[80]



For haplotype analysis we used a marker order which was derived from the NIH-CEPH Collaborative Mapping Group[127], complemented with data from the Généthon group[49;82] and a radiation hybrid map.[48] For all markers, the odds for their mutual order was 1,000:1 unless stated otherwise. The investigation of the inferred second locus for hereditary paragangliomas on 11q13 was performed with the markers D11S554, D11S905, D11S956, D11S480, PYGM, and FGF3.

Results

In an attempt to further map PGL more accurately, a search for recombinants in the disease-associated haplotype was initiated by typing a total of 19 families with the markers D11S897, NCAM, D11S490 and CD3D, which map to both extremes of the 16 cM candidate region reported previously.[91;92] Both linkage and haplotype analysis failed to provide evidence for such recombinants, possibly because many of the kindreds analyzed were of relatively small size, and few patients in at least two generations were available for marker typing. By genealogical analysis, we were able to link two of these families into the kindred in which the initial linkage to 11q22-q23

was reported (FGT01) [91], resulting in a large 7-generation multibranch family derived from a small geographic area in the Netherlands (Figure 5-1). The comparison of the disease haplotypes of all affected individuals would in principle allow the detection of ancestral recombination events. To this end, a total of 190 individuals, including 25 affected subjects, were genotyped at 21 different polymorphic markers mapping at regular intervals across the entire 16 cM candidate region (Figure 5-2).

Linkage analysis

Table 5-1 shows the 2 point lod score calculations for 12 of the 21 markers used. Seven markers provided significant evidence for linkage, but only D11S908 showed linkage at $\Theta = 0.00$ (maximum lod score of 3.99). D11S1327 and D11S1792, which map close to D11S908, showed weak positive scores at $\Theta = 0.00$, presumably because their linked alleles are very frequent in the population (64 and 76%, respectively). The frequency of the linked haplotype defined by these three markers was determined to be 18% in our reference population. By recoding the three marker haplotype to a single 'marker', a lod score of 5.77 was obtained at $\Theta = 0.00$ (data not shown).

TABLE 5-1 Two-point Lod Scores for Family FGT189 (8 liability classes) Using Population Based Allele Frequencies.

Marker[1]		Θ					MAX lod	Θ
		0.000	0.010	0.050	0.100	0.200	0.300	
NCAM	-∞	5.90	6.81	6.66	5.38	3.53	6.83	0.06
DRD2	-∞	0.35	0.96	1.06	0.84	0.47	1.06	0.09
D11S560	-∞	7.93	8.74	8.45	6.90	4.71	8.74	0.05
D11S938	-∞	0.61	1.82	2.03	1.65	0.91	2.03	0.10
D11S4122	-∞	4.29	5.27	5.22	4.19	2.66	5.32	0.06
D11S1792	0.88	0.86	0.79	0.67	0.39	0.14	0.88	0.00
D11S1327	1.42	1.38	1.22	1.03	0.66	0.34	1.42	0.00
D11S908	3.99	3.92	3.60	3.18	2.24	1.28	3.99	0.00
D11S1885	-∞	7.22	7.23	6.61	4.96	3.09	7.36	0.02
D11S2082	-∞	2.86	4.80	5.16	4.51	3.14	5.16	0.10
D11S490	-∞	2.74	2.93	2.61	1.72	0.87	2.96	0.03
CD3D	-∞	2.61	3.58	3.57	2.82	1.74	3.63	0.07

[1]Markers are displayed from centromere to 11q telomere.

Haplotype analysis

A total of 29 affected persons were ascertained in the lower 3 generations, and the disease-linked haplotypes were reconstructed with data from 21 markers in 25 of these (Table 5-2). No recombinants were detected in the disease haplotype in those cases where marker data were available for affected or carrier parents as well as their affected child(ren). Therefore, for the sake of comparison, all sibships with an affected case were considered a separate branch of the family, designated A through K (Figure 5-1), and each could be represented by a single disease-associated

TABLE 5-2 Haplotype Analysis in the Family FGT189.

Marker[1]	Disease haplotype in branch											Freq.[2]	Alleles[3]
	A	B	C	D	E	F	G	H	I	J	K		
D11S876	6	6	6	6	4	4	4	1	7	7	7	6	11
D11S897	2	2	1	1	1	1	1	1	1	1	1	7	8
NCAM	7	5	3	3	3	3	3	3	3	3	3	7	12
DRD2	2	3	2	2	2	2	2	2	2	2	2	52	5
D11S560	8	4	3	3	3	3	3	3	3	3	3	4	8
D11S938	1	2	3	3	3	3	3	3	3	3	3	58	6
D11S4122	7	7	5	5	5	5	5	5	5	5	5	10	7
D11S1792	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**76**	**4**
D11S1327	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**64**	**4**
D11S908	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**38**	**6**
D11S1885	4	2	2	2	2	2	2	2	2	2	2	11	10
D11S1992	2	3	3	3	3	3	3	3	3	3	3	.	6
D11S2077	2	2	1	1	1	1	1	1	1	1	1	.	2
D11S4092	4	4	5	5	3	3	3	3	5	3	3	46	6
D11S939	4	4	4	4	2	2	2	2	2	2	2	45	4
D11S1340	2	2	5	5	2	2	2	2	1	1	1	22	6
APOC3	1	4	5	5	1	1	1	1	9	9	9	2	15
D11S2082	8	4	1	1	10	10	10	10	10	10	10	6	13
D11S721	7	4	4	4	11	11	11	11	11	11	11	.	.
D11S490	4	6	6	6	6	6	6	6	6	6	6	32	11
CD3D	2	4	1	1	4	4	4	4	4	4	4	27	6
Patients[4]	1	2	2	1	1	3	4		8	1	2	3	

[1]Markers are shown from centromere to 11q telomere.
[2]Freq. = Percentage of the most frequent disease-allele among 82 chromosomes marrying into the family. ND = Not determined.
[3]Total number of different alleles within this family.
[4]Number of patients in which the haplotype is found. The marker STMY (previous border) is located between the markers D11S876 and D11S897.

haplotype (Table 5-2).
Since all these branches descend from the same ancestral female, all patients are presumed to carry the same gene defect and alleles of markers closely bordering this gene are expected to be identical by descent. This phenomenon was observed with the same 3 markers that showed tight linkage, i.e. D11S1327, D11S1792, and D11S908 (Table 5-2). This suggests that ancestral recombinants have occurred between PGL and D11S938/D11S4122 (these markers are genetically not separated) and between PGL and D11S1885. These markers span a sex-average genetic distance of 2 cM.[49] Notably, the haplotypes C through K appear identical over a much larger region of chromosome 11q, namely between the markers D11S876 and D11S4092, a distance of approximately 10 cM. Haplotypes A and B are most divergent from this, and share only the region D11S938-D11S1885 between them.
While this work was in progress, a small family of French origin (FGT21) was referred to our lab. After haplotyping, the 2 affected nieces (ID number 8 and 10 in Figure 5-3) appear only to share the region proximal to marker D11S908. This implies a recombination between markers D11S1327 and D11S908 in either one of

the parents. Unfortunately, we do not have DNA samples from the grandmother to determine where this recombination occurred. These results suggest that D11S908 can be excluded from the region containing PGL

Locus heterogeneity

A second locus for PGL has been mapped to 11q13 in a single family between the markers D11S956 and PYGM, a sex average genetic region of 5 cM.[133;134] These and several other markers, including D11S480, the only marker showing complete allele-sharing in that family, were investigated here. No common haplotype could be defined among the branches E-H and among the branches A, C and D (data not shown). Also in family FGT21 no shared haplotype could be detected between the 2 affected individuals, of whom marker D11S480 is shown in Figure 5-3.

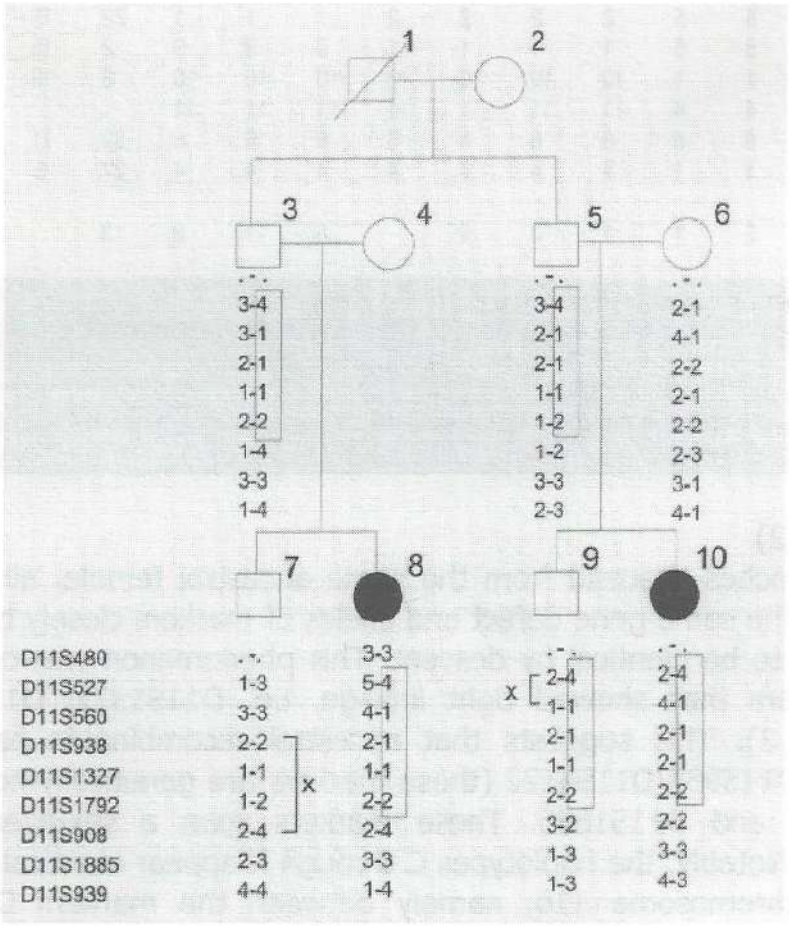


FIGURE 5-3
Pedigree of family FGT21. The symbols used are as in figure 5-1. Chromosome 11q markers are displayed from centromere to telomere. The shared segment of chromosome 11q23 between the two patients is boxed. Marker D11S4122 is not informative in this family.

Discussion

We have presented evidence that PGL maps to a 2 cM interval on 11q22-q23 by haplotype sharing between affected persons in a large Dutch family and a small French family. Both kindreds are consistent with the sex-dependent modification of gene expression ('imprinting'). Recently, genomic imprinting in families with paragangliomas of the head and neck region was also confirmed independently for 9 US families.[138] Our results represent a significant reduction of the candidate gene region, which stood at 16 cM after recombinant analysis of 6 families[49;92], and now excludes the dopamine receptor 2 gene (DRD2) and neural cell adhesion molecule gene (NCAM) as candidates for the disease. The PGL region maps between the markers D11S938/D11S4122 and D11S908/D11S1885. The physical localization of these marker pairs in relation to each other is not yet known.

The order of the markers used for the reconstruction of haplotypes was compiled from different types of maps: genetic maps[1;49;82;127] and a radiation hybrid map.[48], which overlap partially in terms of markers used. One inconsistency of particular concern for this study was the position of D11S1327. RH mapping placed it at the same position as D11S1792[48], which is supported by characterization of YACs in this region [M. James, unpubl.]. Meiotic recombinant analysis in CEPH families, however, located this marker 1 cM proximal to D11S938[49], i.e. 3 cM proximal to D11S1792 and D11S908. On the basis of the depth of the YAC contig at this position, the RH map, our own cosmid map (unpublished), and the potential errors in CEPH family analysis, we have here assumed that D11S1327 and D11S1792 are tightly linked.

A potential pitfall in the analysis of the haplotype sharing might be that all family members are from a small isolated community living in a small geographic area in the Netherlands. Thus an identical haplotype might have been brought into the family by an unsuspected consanguineous relationship. Consequently, certain recombination events might have been missed. Indeed, the two large blocks of shared haplotypes interrupted by a discordant block in branches E-K (Table 5-2) might be reflecting such an event. However, since all affected members of the family carry the same mutation by descent, this can only lead to overestimation of the candidate region, but not to a false candidate region. On the other hand, underestimation of the candidate region could occur by mutations in the markers bordering this region. Since frequencies for such events are low (10^{-3}-10^{-4})[211] and any 2 patients in the large family are at most separated by 14 meiotic events, this seems less likely here. The power of linkage disequilibrium mapping and/or haplotype sharing for gene isolation in founder populations has been demonstrated before.[85;170] Due to the high frequencies of the linked alleles at the three shared loci, the statistical support for this region is just significant. The isolation of additional markers within the current gene region with a low frequency of the disease-linked allele should increase this significance. The localization reported here is supported independently by our analysis of loss of heterozygosity in several glomus tumors, both sporadic and familial.[48] These tumors all seem to affect the region distal to marker D11S560, which includes the currently defined 2-cM candidate gene region.

The absence of meiotic recombinants in 17 families, although they were of various

sizes, is unexpected. At least 50 informative (i.e. of paternal origin) meioses could be scored, and hence about 8 recombination events were expected, although an excess of female over male recombination has been noted in this region.[123] On the other hand, regions as large as 10 cM have been found to be transmitted randomly without recombination through multiple generations in several extended families.[80;95;159] Our inability to identify recombinants in the initial screen of 17 families might thus be purely coincidental, particularly as we found evidence for at least a few ancestral recombination events. It is nonetheless tempting to consider that if recombination suppression existed in this region, it may be related to the disease-causing mutation. A link has been proposed between genomic imprinting and sex-specific recombination[161], while a class of mutations has been proposed that might interfere with the process of genomic imprinting.[32;171] We cannot exclude that the mutation in PGL would render an otherwise non-imprinted gene susceptible for genomic imprinting, concomitantly affecting meiotic recombination.

We were unable to obtain evidence for the involvement of an inferred second locus for PGL at 11q13[133], not in the two families reported here, nor in other families.[92;134](Our unpublished data) This suggests that, if a second exists, it will play a minor role in inherited paraganglioma, but formal heterogeneity analysis will be required to confirm this.

Although our results represent significant progress towards identifying PGL, a genetic distance of 2 cM would imply a physical size of approximately 2 Mb, and therefore a further reduction of this region is still required. A detailed physical map of the region, in conjunction with linkage disequilibrium measurements should enable this.

Chapter 6

Founder Effect in Hereditary Head and Neck Paraganglioma Families

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Introduction

Paragangliomas are rare, usually benign tumors of the extra-adrenal paraganglion tissue associated with the autonomous nervous system.[163] Most paragangliomas occur in the head and neck region, where they may lead to cranial nerve deficit. Characteristically the tumor progresses slowly and although the age of onset is variable, most patients develop symptoms after puberty. Familial nonchromaffin paragangliomas of the head and neck (HN-paragangliomas, MIM 168000) inherit as an autosomal dominant disease with reduced penetrance.[8;138] Affected offspring is observed only after paternal transmission, which has been taken as evidence that the underlying gene-defect is subject to genomic imprinting.[143]

Linkage analysis of a single large Dutch pedigree mapped the gene, termed PGL1, to 11q22-q23.[91] This result was replicated in additional families[92], and confirmed in North American families[17;146], but the detected recombination events did not assign the gene any more accurate than to an approximately 10-cM interval. We recently identified a common ancestor, born in 1776, of three families originating from the same geographical region, including the one in which the original linkage was found. A 2-cM haplotype, presumably containing PGL1, was shared among all patients in the two lowest generations of these families.[184] Although a second locus has been implicated to reside on 11q13 in one other Dutch paraganglioma family[133], all informative families analyzed to date have revealed only linkage evidence for the distal PGL1 locus on 11q22-q23.[17;92;146]

Recently another 10 families with HN-paragangliomas were ascertained from the same geographical region as from which the large PGL1-linked family originated. Assuming that such conspicuous geographic clustering of a rare disorder might reflect a founder effect, which could be exploited for further gene localization, we performed a genealogical survey and determined the disease-linked haplotypes for all 10 families. Although no family relationships could be demonstrated by genealogy, haplotype analysis provided strong evidence for a common founder in this population.

Materials and Methods

Family ascertainment

Since 1950, 183 patients with head and neck paragangliomas were referred to our ENT Department. Queries were sent to those patients with a recorded family history of HN-paragangliomas, enabling us to ascertain 27 pedigrees with at least 142 patients. Three families (FGT1, FGT8, FGT9) originating from the central western part of the Netherlands could be traced to a common ancestor[184], and was renamed FGT189. Fourteen other families originated from the same province as did family FGT189, and we obtained DNA samples to reconstruct the disease haplotype from 10 of these. FGT3, FGT11 and FGT18 were partially described previously[84;92;143], others are reported here for the first time.

Disease ascertainment

Diagnosis of HN-paragangliomas was based on clinical signs and in most patients

confirmed by histological or radiological investigation. Twelve probable affected progenitors were identified by evaluating their medical history. Ten of these putative patients, of whom two died during surgical intervention, are known to have had lateral neck masses. The other two are known to have had ear complaints leading to bleeding or loss of facial nerve function.

Genealogy analysis

Familial ancestries were traced back starting from the oldest known common ancestor of affected family members, and included both the paternal and maternal line. Most data were obtained from the civil registration founded in approximately 1800. Generations older than this were not studied. Three ancestral lines were not completed: in family FGT27 data on one generation in the maternal line was not available; in family FGT32 an in-marrying spouse born approximately 1850 could not be traced; in FGT20 the genealogical search was thwarted by an adopted ancestor. The Dutch fore-bearers were usually farmers or handicraftsmen; marriages outside the native village were common but little migration to other regions was observed until after World War II.

DNA isolation and PCR analysis

Blood samples were collected and genomic DNA was isolated from peripheral blood lymphocytes.[145] PCR and gel-electrophoresis conditions to visualize microsatellite polymorphisms were as described previously.[184] All primer sequences for these markers are retrievable online from the Genome Data Base, and all oligonucleotides were manufactured by Isogen Inc. (Maarssen, The Netherlands).

Table 6-1 Clinical data from 10 HN-paraganglioma kindreds

Family	Number of Patients			Tumors[c]				Religion[d]
	Total[a]	Verified[b]	Genotyped	CBT	VBT	JTT	PH	
FGT3	13	8	4	6	4	7	0	RC
FGT5	5	4	3	5	2	0	0	P
FGT11	2	2	2	1	0	2	0	P
FGT17	7	4	2	3	0	2	1	RC
FGT18	18	13	12	15	2	0	0	P
FGT20	3	2	2	4	3	1	1	P
FGT25	7	5	5	4	1	1	0	RC
FGT27	4	1	2	1	0	0	1	RC
FGT29	2	1	2	1	0	1	0	P
FGT32	2	2	1	2	3	3	0	RC
Totals	63	42	35	42	15	17	3	

[a] Total number of patients identified anamnestically
[b] As documented by medical records
[c] CBT = carotid body tumor, VBT = vagal body tumor, JTT = jugulo-tympanicum tumor, PH = pheochromocytoma; [d] RC = roman catholic, P = protestant

Haplotype analysis

For haplotype analysis we used a marker order as described by van Schothorst et al.[184] The genetic map NIH-CEPH Collaborative Mapping Group, 1992;[49;127] was complemented with data obtained by physical mapping of the region between markers D11S897 and D11S4111.[20] The markers selected covered a genetic distance of approximately 50 cM. Allele lengths were determined using an M13-sequence as reference. Allele frequencies in the control population were determined in 20 in-marrying spouses of family FGT189, and 21 unrelated members of families with the familial atypical multiple mole melanoma syndrome, and originating from the same area as FGT189.[80] Allele-lengths and frequencies thus determined were not appreciably different from those reported in the Genome Data Base.

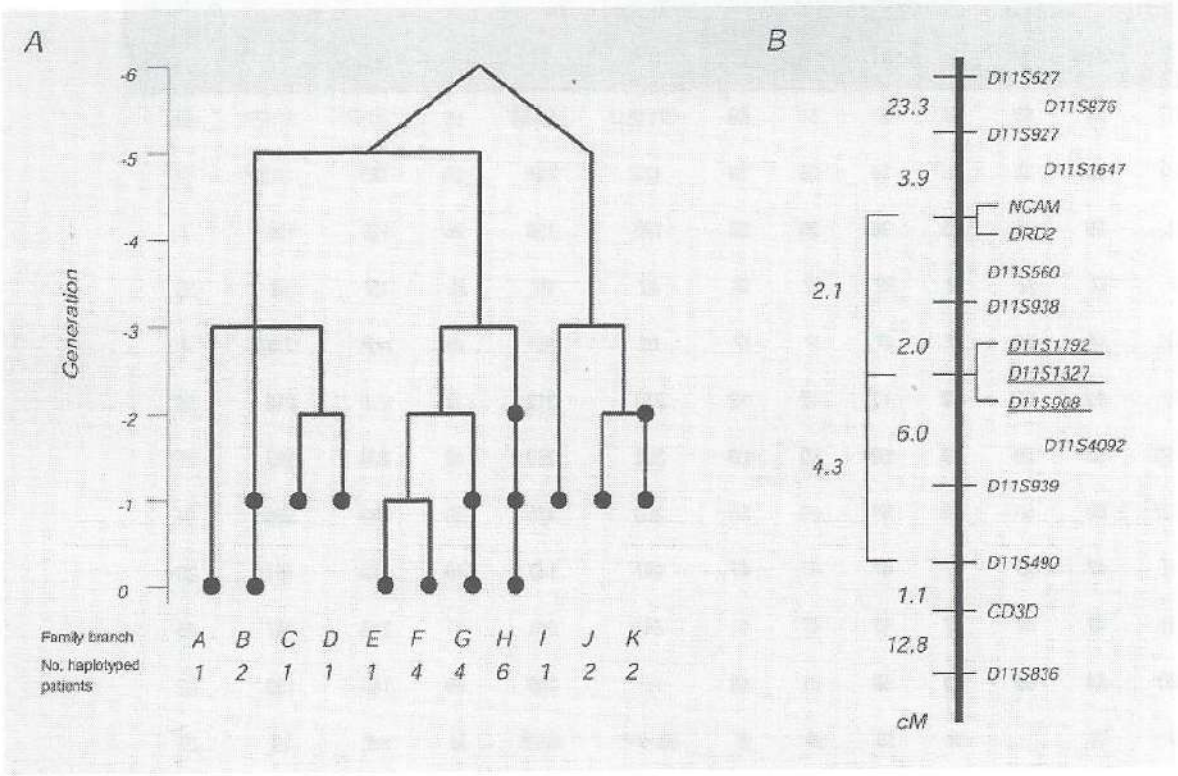

FIGURE 6-1

A. Abbrieviated version of the pedigree structure of extended family FGT189, described in detail in the study by van Schothorst et al. (1996).[184] The family was arbitrarily subdivided into branches A-K. Blackened cercles represent sibships in which at least one patient has been ascertained. The number of halotyped patient is indicated below each branch.

B. Genetic map of markers used in this study. Distances are in centimorgans (cM). The underlined markers showed allele sharing in all patients of family FGT189. The markers in italics are provided for orientation.

Results

Clinical description and inheritance patterns in 10 HN-paraganglioma families

A total of 63 HN-paraganglioma patients were identified in the 10 families presented here, 42 with complete medical records (Table 6-1). The carotid bifurcation was the most frequently affected site (57% of all HN-paragangliomas). Multiple paragangliomas occurred in 66% of the patients, as expected for inherited cases.[138] Three

patients in different families developed a paraganglioma of the adrenal gland (pheochromocytoma). In family FGT11, primary hypothyroidism occurred in a father and his daughter, who both also had HN-paragangliomas. The pedigrees were consistent with the hypothesis of genomic imprinting of PGL1.[143] No affected offspring was observed from female carriers and all affected family members received the disease gene from their father. Remarkably, 42 patients received the gene from their grandfather whereas only 2 patients received it from their grandmother (for 19 patients the transmitting grandparent could not be determined).

TABLE 6-2
Disease Haplotypes of Branches E-H of Family FGT189 and 10 Other Families from the Same Region

Marker	Linked allele-size (bp) in family FGT											Frequency (%)[a]
	18?	11	18	20	32	25	3	17	5	29	27	
D11S527	59	59	47	57	51	59	57+151[b]	5/159[c]	65	7/157[c]	1/155[c]	16
D11S927	35	35	35	35	35	35	135	135	35	135	1/137[c]	18
NCAM	26	26	26	26	26	26	126	126	26	126	126	7
DRD2	32	32	32	32	32	32	82	82	32	82	82	52
D11S560	37	37	37	37	37	37	87	nd[d]	87	nd[d]	7/87	4
D11S938	12	12	12	12	12	12	212	212	12	212	212	58
D11S1792	69	69	69	69	69	69	269	269	69	269	269	76
D11S1327	50	50	50	50	50	50	250	250	50	250	250	64
D11S908	47	47	47	47	47	47	147	147	49	149	151	38
D11S939	47	47	47	47	47	41	241	241	41	249	247	45
D11S490	59	59	59	59	61	49	167	159	59	149	159	32
CD3D	39		39	39	39	35	93+91[b]	9/93[c]	39	nd[d]	89	27
D11S836	70	72	72	74	74	72	74+66[b]	74	74	4/70[c]	74	8

Families are ordered according to the extent of haplotype sharing with family FGT189.
[a] Frequency of alleles which define the 'E/H-haplotype' in FGT189 (shaded) in control population
[b] A recombination event was observed in this family
[c] Phase unknown
[d] Not determined

Haplotype analysis

Blood samples were obtained from 136 family members, including 35 patients (Table 6-1). DNA was genotyped at 13 markers encompassing an approximately 50-cM interval on 11q13-q24. Previously, we identified a 3-marker haplotype of about 2 cM, defined by markers D11S1792, D11S1327 and D11S908, which was conserved among all the patients from a large multibranch family FGT189 (summarized in figure 6-1).

However, a much larger haplotype of approximately 10 cM, defined by markers between D11S876 and D11S4092, was shared among the patients from branches C-K, and this sharing extended beyond CD3D in branches E-H. We compared this 'E/H-haplotype' with the disease-linked haplotypes of the 10 families included in this study (Table 6-2). No recombinants were detected between the disease and any marker mapping between D11S1647 and D11S908, which were previously shown to be recombinant in two independent families.[17;184] All patients shared a haplotype defined by 6 markers and bracketed by D11S927 and D11S908. This haplotype was not observed among 41 unrelated individuals (82 chromosomes) from the same geographical region, supporting the hypothesis that all patients in these 11 families are genetically identical by descent. Notably, several families, i.e., FGT11, FGT18 and FGT20, appear to share a very large region with family FGT189, including all but the most distant markers tested.

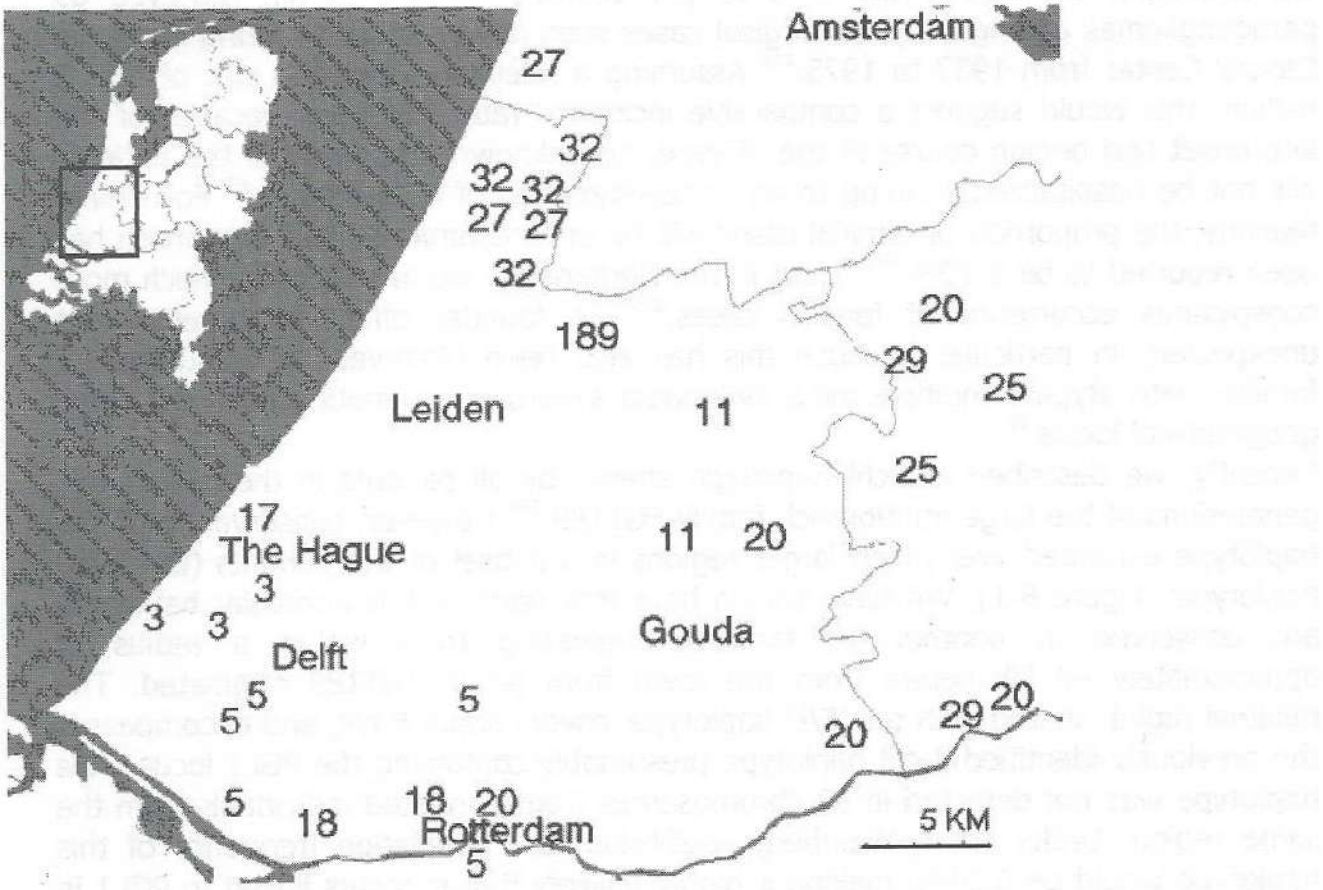

FIGURE 6-2
Map of the studied region. The numbers indicate the birthplaces of the 18th century ancestors of the corresponding families. Only ancestors of the oldest patient's father are included. The inset shows a full map of the Netherlands.

Genealogy

Records on 72 ancestors of the 10 families were retrievable. They were born between 1770 AD and 1830 AD, and originated from several rural areas of the central western part of the Netherlands, all within a radius of approximately 40 kilometers (Figure 6-2). Half of the families belonged to a Protestant church whereas the others were of Roman Catholic faith (Table 6-1). No marriages between members of different convictions were observed. Despite the strong geographical clustering and this sharing of religious faith, none of the studied families could be proven to be interrelated.

Discussion

Paragangliomas of the head and neck usually follow a slow benign natural course, and generally occur above the age of 18.[8;69;163] As a result, they are expected not to impede reproductive fitness. From pooled data from Dutch pathological laboratories, we estimated an annual rate of 0.11 per 100,000.[160] Lack et al., retrieved 69 paragangliomas among 600,000 surgical cases seen at the Sloan-Kettering Memorial Cancer Center from 1937 to 1975.[116] Assuming a relevant population size of 1 to 2 million, this would suggest a comparable incidence rate. However, because of the late onset and benign course of the disease, an unknown proportion of the patients will not be hospitalized, leading to an underestimation of its incidence.[69] For similar reasons, the proportion of familial cases will be underestimated. This proportion has been reported to be 5-10%[79;163], but in the Netherlands we are seeing a much more conspicuous occurrence of familial cases.[8;143] A founder effect is therefore not unexpected, in particular because this has also been observed for a number of families with atypical multiple mole melanoma syndrome originating from the same geographical locale.[80]

Recently, we described a 2-cM haplotype shared by all patients in the lowest two generations of the large multibranch family FGT189.[184] However, conservation of this haplotype extended over much larger regions in a subset of the patients (the 'E/H-haplotype', Figure 6-1). We have shown here that parts of this particular haplotype are conserved in another 10 families originating from within a radius of approximately 40 kilometers from the town from which FGT189 originated. The minimal region shared with the 'E/H-haplotype' covers about 6 cM, and encompasses the previously identified 2-cM haplotype presumably containing the PGL1 locus. This haplotype was not detected in 82 chromosomes from unrelated individuals from the same region. Under Hardy-Weinberg equilibrium the population frequency of this haplotype would be 0.04%, making it highly unlikely that it occurs linked to PGL1 in 11 families by chance. Since we included 10 of the 14 HN-paraganglioma families ascertained from this region, these data therefore strongly indicate that a single ancestral mutation in PGL1 is responsible for most paragangliomas occurring in the central western part of the Netherlands.

Genealogy of these 10 families was not able to link any of them to FGT189, suggesting that this mutation must at least be 200 years old. The absence of assimilation between families of Protestant or Roman Catholic faith even suggests

that the common ancestor lived before the Reformation in the 16th century.
In the light of the age of this PGL1-mutation, it is remarkable that several families (FGT11, FGT18, FGT20) share a region of over 15 cM with the 'E/H-haplotype' of FGT189. In families FGT11, FGT25, and possibly FGT17, the haplotype conservation may even extend proximally to include marker D11S527, mapping to subband 11q13.5, although this might be coincidental given the frequency of the shared allele at this marker. The genetic distance between the haplotyped patients from families FGT11, FGT18, and FGT20, and from the branches E-H of family FGT189, must be at least 16 meioses. We have suggested a deficit of recombination-events involving the disease-linked haplotype in paraganglioma families[184], but this lacks statistical support so far. In fact, such events must have occurred more recently in branches A and B of family FGT189, as these confine the haplotype sharing to 2 cM. Moreover, a more than two-fold excess of female versus male recombination has been reported for this region of chromosome 11.[127] Thus the size of the conserved haplotype might partly be explained by the over-representation of male transmission in our families. Finally, strong haplotype conservation has been reported in other founder populations as well, and might not be uncommon.[165] A 10-cM haplotype has been reported to be conserved for 450 years in Finnish families with hereditary non-polyposis colorectal cancer.[159] Nevertheless, the finding that genomic imprinting may interfere with sex-specific recombination rates[161;172] makes it tempting to speculate that the mutation in PGL1 responsible for HN-paragangliomas also affects recombination rates in this region of chromosome 11.
The over-representation of paternal and grand-paternal transmission we have noted here might be explained by an ascertainment bias owing to the fact that HN-paragangliomas develop only after paternal transmission.[143] Even though PGL1-carrying females would on average have the same chance of having affected grandchildren as PGL1-carrying males, their affected grandchildren are less likely recognized as hereditary cases because they derive the gene from their non-penetrant father. This would imply that an unknown proportion of allegedly sporadic paraganglioma patients are in fact hereditary.
Another interesting feature of this founder mutation in PGL1 is its apparent ability to predispose to pheochromocytomas (the paraganglioma of the adrenal gland). We detected 5 cases of this rare tumor in the 11 families studied here (table 6-1 and[69]), confirming earlier suggestions of an association with HN-paragangliomas.[27;179] PGL1 might thus be another factor in the already heterogeneous genetic basis of familial pheochromocytomas.[215]
Our finding of a strong founder effect at PGL1 contrasts with haplotype analysis of North American HN-paraganglioma families, in which no obvious, large regions of allele sharing were apparent.[17] Two families of distant Polish ancestry shared alleles for D11S938, D11S1792, and D11S1327 in that study, the latter two of which map to the 2-cM region defined by haplotype sharing in family FGT189. Yet the population frequency of 0.30 of this haplotype precluded an unambiguous conclusion of a common origin. A comparable situation exists for the 2-cM haplotype shared in FGT189, for which the population frequency was determined to be 0.18[184], but for

which the common ancestor was identified genealogically. The founder effect reported here can now be exploited further for gene-mapping purposes, by analyzing linkage disequilibrium across a closely spaced, highly polymorphic marker-map covering the shared 11q23 region and its immediate flanking regions, in more extended series of HN-paraganglioma patients of Dutch origin.

Chapter 7

Two Founder Mutations in the SDHD gene

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Introduction

Hereditary paragangliomas (MIM 168000) are rare, usually benign tumors of neuroectodermal origin. The majority of these tumors arise from the paraganglionic bodies in the head and neck that have a chemoreceptive function and that arise in close relationship to the parasympathetic nerves and large vessels. Tumor growth is generally slow, but may cause severe morbidity.[18] The tumors consist of two cell types: nests of large chief cells surrounded by elongated sustentacular cells. The cell nests are separated by a highly vascular stroma. The incidence of paragangliomas has been estimated to be 1 in 100,000-1,000,000.[19] The age of onset is between 14 and 65 years. Paragangliomas only develop after paternal transmission of the disease-causing mutation; maternal transmission remains non-penetrant. This has been taken as evidence that the expression of the gene defect underlying paragangliomas is modified by genomic imprinting.[29]

Hereditary paragangliomas are genetically heterogeneous. Two loci, PGL1 and PGL2, have been mapped to chromosome bands 11q22-q23 and 11q13, respectively.[91;133] Exclusion of these loci in a German family suggests the existence of a third locus.[156] Previously, haplotype analysis suggested that 73% of the Dutch patients shared the same founder mutation.[185] Recently, the SDHD gene was identified as the PGL1 candidate gene using positional cloning methods.[18] The SDHD gene encodes the small subunit D of cytochrome b558 of the mitochondrial respiratory chain complex II (succinate: ubiquinone oxidoreductase). The aim of this study was to confirm the presence of SDHD mutations on the founder haplotypes and to identify additional mutations in our collection of paraganglioma families and isolated patients. In addition, we demonstrate that the SDHD gene is involved in the non-random loss of heterozygosity (LOH) on chromosome 11q22-23, which has previously been observed at distant flanking markers in paragangliomas.[48;183] This non-random LOH identifies SDHD as the first tumor suppressor gene encoding a mitochondrial protein.

Materials and Methods

Patients and controls

Patients with hereditary paraganglioma in the head and neck region (tumors arisen from the glomus caroticum, glomus jugulare, glomus tympanicum, and glomus vagale) were selected on the basis of their medical history, physical, radiological, and otorhinolaryngological examination. Cases were considered to be familial when at least two affected individuals were identified, preferably in more than one generation. Genealogy was extended to include at least the grandparents of the probands. Isolated cases are defined as having no family history for head and neck paraganglioma in the parental and grandparental generations.

Mutation analysis was performed in 98 unrelated patients: 41 patients from independently ascertained families and 58 isolated patients. Thirty-two familial patients were from the Netherlands, 4 from the USA, and 1 each from Belgium, France, Italy, and Canada. In the 32 Dutch families, 135 patients have been ascertained. Fifty-five isolated patients were of Dutch, and 3 of British origin. Twelve of the Dutch families have been described previously.[133;185] Forty unrelated healthy individuals from the

Western part of the Netherlands were included as controls. Informed consent was obtained from all the patients in this study, which was approved by the Leiden University Medical Center IRB.
Genomic DNA samples were prepared from peripheral blood or from EBV-transformed lymphocyte cell lines by standard procedures.[145] Tumor DNA was isolated from sorted paraganglioma tumor cells as described previously.[184]

PCR conditions
Single exon PCR analysis was carried out on 100 ng genomic DNA in a total volume of 25 µl at a final concentration of 10mM Tris-HCl (pH 8.8), 75 mM KCl, 1.5 mM $MgCl_2$, 200 µM each dNTP, 0.025 U/µl of E-Taq polymerase (Eurogentec, Seraing, Belgium), in the presence of 10 pmol of exon-flanking primers (Table 7-1). Denaturation was 3 min at 94°C, followed by 35 cycles of amplification with denaturation for 1 min at 94°C, annealing for 2 min at 58°C, and extension for 1 min at 72°C, with a final extension for 5 min. Twenty µl of PCR sample was analyzed on a 2% agarose gel. Polymorphic markers D11S5014, pDJ-GT1, pDJ-CA, D11S5019, and D11S4078 were amplified as described previously.[18;19;49]

TABLE 7-1 SDHD Primers Used for Mutation Detection

Exon	Forward primer (5'> 3')	Reverse primer (3'> 5')	Length (bp)
1	gttcacccagcatttcctctt	tgctgtgatttcggtattttc	281
2	atgttatcccctatttattgtt	tctgcccaaaggtgtaaacta	375
3	cattgagataccttgtgctaa	ttcaatcaacttctccctcata	387
4	gtggagtggcaaatggagaca	tctgttatttcttccttattgtga	463

SSCP analysis
Exon products were amplified in the presence of α-^{32}P-dCTP and analyzed on 0.5 MDE (FMC), 5%, and 10% polyacrylamide plus glycerol gels, which were run overnight at 20 Watt and room temperature. The conformers were visualized by overnight exposure on X-ray films (Eastman Kodak Company, Rochester, NY).

DNA sequence analysis
Amplified exon products were purified by use of the Qiaquick PCR purification kit (Qiagen, Valencia, CA). The exons were sequenced on the ABI 377 sequencer using the same exon amplification primers and the Big Dye Terminator cycle sequencing kit (Perkin Elmer) in both directions.

Mutation detection by Restriction Endonuclease Digestion
Amplified exon products were digested with the relevant restriction enzyme according to the recommendation of the manufacturer. Each sample was analyzed on a 2% agarose gel, stained with ethidium bromide, and visualized by an UV transilluminator.

Results

Mutation analysis

In all 41 families studied here, the disease displayed exclusive paternal inheritance. We screened one patient from each family, and all isolated patients for mutations in the SDHD gene by single strand conformation (SSCP) analysis. PCR products with aberrant migration were sequenced. Seven novel SDHD mutations and one putative polymorphism were identified in addition to the five mutations and one polymorphism reported previously by Baysal et al. (Table 7-2).[18] Most SDHD mutations result in the loss or gain of a restriction site and can be detected by restriction enzyme digestion of the PCR products. In each family, the mutation co-segregated with the previously determined disease haplotype (data not shown). None of the SDHD mutations were found in DNA from 50 individuals.

TABLE 7.2 Mutations Identified in SDHD

SDHD mutations	Exon	Protein	Families with mutation	Isolated patients with mutation	Restriction digestion
g.6759 insC[1]	2	frameshift after A18	1 USA		AluI loss
g.6769 C>T[1]	2	R22X	1 Can		BstBI loss
g.6811 C>T[2]	2	Q36X	0		MboII loss
g.6817 C>T[2]	2	R38X	1 USA		AvaII loss
g.6825 insC[1]	2	frameshift after P41	1 NL		BsII gain
g.7806 A>G[1]	3	R70G	1 B		none
g.7840 C>T[2]	3	P81L	1 UK, 1 USA	3 UK	MspI loss
g.7872 G>T[2]	3	D92Y	23 NL	19 NL	RsaI gain
g.7882 T>C[1]	3	L95P	0	1 NL	MspI gain
g.7903 A>T[2]	3	H102L	0		NlaIII loss
g.13678 T>C[1]	IVS3 -32 T>C	?	0	1 NL	none
g.13811 T>C[1]	4	L139P	6 NL	1 NL	HaeIII gain
SDHD polymorphisms					
g.5842 G>A[1]	1	G12S	1 B		NlaIV loss
g.7802 C>T[2]	3	S68S	0	1 NL	SpeI gain

[1]present study [2]Baysal et al. (2000)[18]

Founder mutations in familial cases

Two mutations, Asp92Tyr and Leu139Pro, were identified in 24 and 6 Dutch paraganglioma families, respectively (Table 7-2). The Asp92Tyr mutation resides on the founder haplotype that was defined previously by polymorphic markers flanking the PGL1 locus in 11 families.[185] The mutation causing a frameshift after Pro41 was found in a single Dutch family. Together, these three mutations account for 119 of the 135 Dutch familial paraganglioma cases. Two novel protein-truncating mutations were found in exon 2 in two families of North American and Canadian origin, respectively. In a Belgian family, two amino acid substitutions, Gly12Ser in the mitochondrial signal

peptide and Arg70Gly in the mature protein, and the Ser68Ser polymorphism were co-segregating with paragangliomas. The Gly12Ser substitution and the Ser68Ser changes have also been observed in homozygous state in an imprinted carrier, suggesting that they are polymorphisms (data not shown).

Mutations in isolated cases

We detected the Asp92Tyr founder mutation in 19, and the Leu139Pro in one of the 55 Dutch isolated patients (Table 7-2). Furthermore, 2 distinct SDHD germline mutations were found in 2 additional patients. One of these, the nucleotide substitution in intron 3 at position –32, could change the intron 3 branching site. No RNA was available to determine whether this sequence change results in abnormal splicing of the SDHD primary transcript and is a disease-causing mutation. Three isolated patients from the UK carried another founder mutation, Pro81Leu, which has been detected before in 5 American families of English descent.[18] Clinical examination had revealed multiple paragangliomas in 10 of the 55 Dutch patients, and a germline SDHD mutation was present in 8 of these. The relation between the presence of germline mutations and multicentricity is statistically significant according to Fisher's two-sided exact test (p-value 0.01). Of note, SDHD mutations were not detectable by SSCP and direct sequencing in 31 isolated patients with a single tumor.

Genetic heterogeneity

Previously, haplotype analysis has indicated that hereditary paraganglioma in a single Dutch family (Family FGT2) was linked to the PGL2 locus on chromosome 11q13.[133] We have investigated the SDHD gene in three patients from different branches of this family to exclude the possibility that two or more different mutations are segregating within this family. No SDHD mutations were detected by bi-directional sequencing of all exons or by inter-exon PCR using different combinations of SDHD forward and reverse primers. The absence of SDHD mutations in family FGT2 is consistent with the lack of common haplotypes for polymorphic markers flanking the SDHD gene and confirms the genetic heterogeneity of head and neck paraganglioma. Mutations were also not found in three families from France, Italy, and the USA, in which linkage to the SDHD locus could neither be confirmed nor excluded (due to either the small family size or the lack of clinical confirmation of the health status of several family members).

LOH analysis

Previously, we have reported partial loss of the maternally derived allele at polymorphic markers in the SDHD region on chromosome 11q22-q23 in DNA isolated from total paragangliomas.[183] Complete loss was observed in microdissected chief cells and in flow-sorted aneuploid tumor cells. Here, we have used the SDHD mutations as markers in addition to 6 polymorphic dinucleotide repeat markers within 500 kb from the gene to determine whether the SDHD gene itself is the target of the somatic loss of the chromosome 11q22-q23 region. We observed allelic imbalance for the informative dinucleotide repeat markers in 5 out of 7 DNA samples isolated from total paraganglioma tumors (Table 7-3). In all five cases, the intensity of the maternal allele was

reduced with respect to the paternal allele, suggesting that the maternal allele is preferentially lost. The incomplete loss may be caused by the presence of non-tumor cells in the total tumor. Both the normal maternal and the mutated paternal SDHD allele could be amplified from total tumor DNA, but the ratio between the alleles could not reliably be determined, because the intensity of the ethidium bromide staining of the restriction fragments is size-dependent. We therefore repeated the analysis using DNA from flow-sorted diploid and aneuploid cell fractions from 4 different eligible tumors. Near-complete loss of the maternal allele of all informative dinucleotide repeat markers was observed in all aneuploid fractions, but not in the corresponding diploid fractions (Table 7-3). Likewise, complete loss of the wildtype maternal SDHD allele was found in all four aneuploid tumor fractions (Table 7-3, Figure 7-1). The preferential loss of the normal maternal allele suggests that the maternal SDHD allele is expressed in paraganglia cells and that loss of the maternal allele provides a selective growth advantage in the development of paraganglioma.

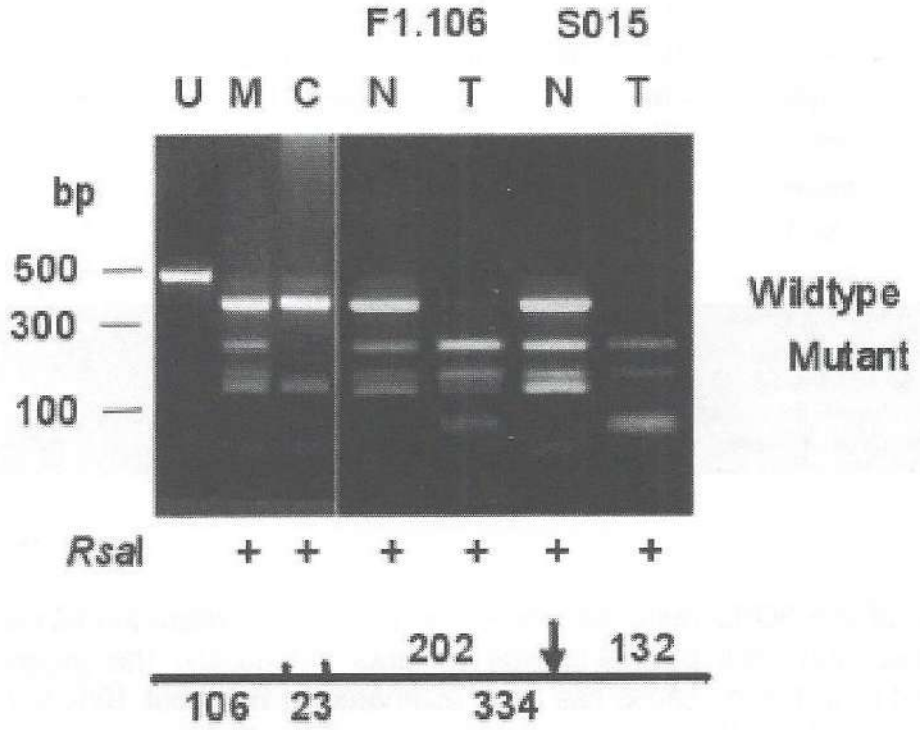

FIGURE 7-1
Loss of heterozygosity of SDHD in sorted tumor cells from paraganglioma patients F1.106 and S015. After PCR amplification, SDHD exon 3 was digested with the restriction enzyme RsaI to detect the presence of the Asp92Tyr mutation. The 463 bp PCR product (U) is normally cut in fragments of 23, 106, and 334 bp (C). The Asp92Tyr mutation creates an extra RsaI site (indicated by the arrow) within the 334 bp fragment. This fragment is cut in fragments of 132 and 202 bp in mutation carrier M. In flow-sorted tumor cells (T), the 334 bp fragment from the normal allele which is present in DNA from normal lymphocytes (N) is lost.

TABLE 7-3 Loss of Heterozygosity in Paraganglioma

			Marker allele ratio					Marker allele ratio	
	Mutation	Tumor fraction	D11S5014	pDJ-GT1	pDJ-CA	SDHD allele	D11S5018	D11S5019	D11S4078
Total tumors						observed			
FGT8.114	Asp92Tyr	total	1.8	1.6	2.7	wt and mutant	NI	1.9	NI
FGT10.122	Leu139Pro	total	1.4	NI	NI	wt and mutant	1.2	NI	NI
FGT15.1	Asp92Tyr	total	NI	NI	NI	wt and mutant	1.5	NI	1.6
FGT17.3	Asp92Tyr	total	1.3	1.1	3.0	wt and mutant	NI	1.3	NI
FGT18.111	Asp92Tyr	total	1.8	1.2	2.0	wt and mutant	1.7	1.7	1.5
FGT18.115	Asp92Tyr	total	NI	NI	NI	wt and mutant	NI	NI	NI
FGT20.118	Asp92Tyr	total	1.3	1.0	1.3	wt and mutant	NI	NI	1.0
Flow-sorted tumors									
FGT1.106	Asp92Tyr	diploid	NI	NI	NI	wt and mutant	NI	F	F
		aneuploid	NI	NI	NI	mutant	NI	>=10	>=10
FGT10.121	Leu139Pro	diploid	1.1	NI	NI	wt and mutant	1.7	NI	NI
		aneuploid	>=10	NI	NI	mutant	4.6	NI	NI
S015	Asp92Tyr	diploid	1.1	NI	NI	wt and mutant	NI	1.1	NI
		aneuploid	>=10	NI	NI	mutant	NI	>=10	NI
S028	Asp92Tyr	diploid	1.7	F	1.1	wt and mutant	NI	NI	F
		aneuploid	>=10	>=10	>=10	mutant	NI	NI	3.0

Marker order is from centromere to telomere
Marker allele ratio: ratio between paternal and maternal alleles for each polymorphic marker.
Allelic inbalance: ratio between 1.5 and 2; LOH: ratio >= 2
The presence of SDHD alleles was determined using mutation-specific restriction digests of PCR products.
Wt = wildtype; NI = not informative; F = failed

Discussion

The identification of the SDHD gene allowed us to assess the proportion of hereditary cases among head and neck paraganglioma patients. Previously, the proportion of patients attributed to a genetic cause has been estimated to be about 10% in the US[79] and 50% in the Netherlands[143], on the basis of recorded family history only. We have collected 190 Dutch paraganglioma patients: 135 patients from 32 families and 55 patients without a detectable family history. Two SDHD mutations, Asp92Tyr and Leu139Pro, were shown to be strong founder mutations in the Netherlands, together explaining the disease in 30 of the 32 families. Another mutation was found in an additional family, whereas no mutations were detected in family FGT2, in which paraganglioma was previously shown to be linked to the PGL2 locus.[133;134] Thus, SDHD mutations explain the inheritance of paraganglioma in 97% of the Dutch families, but the lack of mutations in FGT2 confirms the genetically heterogeneous basis of this disease. The significance of the founder mutations is also underscored by the fact that

we found disease-associated mutations in only 3 of 8 families of non-Dutch origin. Further work should clarify whether this is due to unknown SDHD mutations missed by the technology used here, the absence of a founder effect, or a more significant involvement of PGL2 or SDHC than among the Dutch.

Maternal transmission and genomic imprinting can have masked the hereditary nature of paraganglioma in isolated cases. Although no parents were available to determine whether germline transmission or a *de novo* mutation event occurred, the nature of the mutations suggests that the founder effect at *SDHD* among the Dutch paraganglioma patients is also evident among the 55 non-familial (isolated) cases. A total of 22 (40%) were found to be carrier, of which 20 carried either one of the founder mutations. The mutations in the two remaining patients may reflect *de novo* mutation events. The founder effect explains why the proportion of familial paraganglioma in the Netherlands is much higher than found elsewhere, although some underreporting is expected due to the atypical pattern of inheritance. Germline mutations in a tumor suppressor gene often result in the development of multiple tumors. Multicentricity has been reported to occur in at about 30% of the familial paraganglioma cases.[79;143] Accordingly, we found a strong association between multicentricity and the presence of a germline SDHD mutation among isolated patients. In the 33 remaining patients, paragangliomas could be caused by mutations in PGL2 or SDHC, but this seems less likely given the low proportion of Dutch families due to this gene. Rather, somatically acquired mutations in SDHD or other genes may have caused the disease.

Our results from the SDHD mutation analyses have immediate ramifications for genetic counseling in Dutch paraganglioma patients, as virtually all positively identified familial cases will be due to SDHD. In patients without a family history, the probability to detect a mutation decreases to 40%, but is much higher among those with multiple paragangliomas. Patients with germline mutations are not only at risk to develop multiple tumors, but also may transmit the mutation to their offspring.

Until now, only a few mutations had been identified in the approximately 70 nuclear genes encoding subunits of the complexes. The 14 different SDHD mutations listed here provide the largest body of information on functionally important amino acid residues in mitochondrial respiratory chain proteins. The nature of SDHD mutations differs between the exons (Table 7-2). Exon 1 and most of exon 2 encode the SDHD signal peptide. The mature SDHD protein is encoded by the last 6 bp of exon 2, exon 3, and exon 4. All mutations in exon 2 are truncating, whereas all mutations in exons 3 and 4 are missense mutations in conserved amino acids. Missense mutations may not significantly affect the function of the signal peptide, since mitochondrial signal peptides lack sequence conservation. Therefore, we expect that the Gly12Ser missense mutation in exon 1 is a polymorphism and that the substitution of the conserved Arg70 residue with glycine, which co-segregates with it in a Belgian family, is the disease-causing mutation.[65] Previously, only seven mutations have been detected in five of the complex I (NADH: ubiquinone oxidoreductase) genes[190] and one in the SDHA gene which encodes the flavoprotein subunit of complex II (succinate: ubiquinone oxidoreductase).[29] In sharp contrast to the relatively benign paragangliomas, however, these mutations in homozygous or compound heterozygous state result in severe

respiratory chain deficiencies. Whether homozygosity for SDHD or SDHC mutations can lead to such a severe phenotype remains to be determined.
Paragangliomas only develop after paternal transmission of an SDHD mutation. This pattern of inheritance is consistent with genomic imprinting.[143] Therefore, we expected that the expression of the SDHD gene was regulated by a mechanism involving gene inactivation by differential methylation as has been observed in the Prader-Willi and Angelman syndromes.[32;32] However, differential methylation or allele-specific expression has not been observed for SDHD in different normal tissues.[18] Nonetheless, imprinting of the SDHD region could be limited specifically to paraganglia in the head and neck region, but this could not be determined because normal human paraganglion tissue is very difficult to obtain. Any model for the development of paraganglioma would have to explain the specific inheritance pattern and the loss of the maternal allele in paraganglioma. The loss of the normal maternal allele in tumors from patients is in keeping with the "classical" two-hit inactivation of a tumor suppressor gene and suggests that this allele is expressed. Baysal *et al.* have suggested that the SDHD gene is not completely imprinted in normal paraganglia or that secondary relaxation of imprinting occurs prior to tumor formation.[18] Equally speculative is a model in which the generation skipping of the disease in families is due to a combination of the unique maternal inheritance of mitochondria, and the difference of mitochondrial dynamics between paternal and maternal germline development. Clearly, an SDHD mutation can be transmitted by the oocyte, even though ATP production in its mitochondria is predicted to be 50% less efficient. The number of mitochondria increases 100-fold during oogenesis, but there is a decrease in the number of copies of mtDNA per organelle.[37] Mitochondria do not replicate until the blastocyst stage of embryonal development.[167] Furthermore, of the estimated 2 million primary oocytes in the ovaries of a normal newborn female infant, only ~400 become secondary oocytes, which are expelled at ovulation. Although we know little about the oxidative requirements of primary and secondary oocytes, it is conceivable that those carrying an SDHD mutation can only escape this developmental elimination if they restore ATP production to original levels. This "rescue" might involve a genetic mechanism that persists throughout adult life, effectively suppressing the development of paraganglioma.

Chapter 8

Prevalence of Hereditary Paragangliomas of the Head and Neck

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Head and Neck Paragangliomas; Often a Hereditary Disease?
Jansen JC, Zwinderman AH, Taschner PEM, Schothorst van EM, Devilee P, Grote JJ, van der Mey AGL Cornelisse CJ.

Introduction

Head and neck paragangliomas (synonym: chemodectoma, glomus tumor) are rare tumors that occur in decreasing frequency at the carotid bifurcation, the vagal ganglions and the jugular bulb. Although these paragangliomas usually grow slowly and rarely metastasize, they eventually cause debilitating symptoms due to local expansion. Small tumors can be removed from the carotid bifurcation or tympanic cavity, with few complications. Vagal body tumors cannot be extirpated without sacrifice of the vagal nerve.[43] Cranial nerve paralysis is also common after surgery for large carotid body tumors and paragangliomas in the jugular foramen.[77;83]

Hereditary head and neck paragangliomas (H&N paragangliomas, MIM 168000) reportedly comprise 10% of the total number of cases.[138] In most cases the disease is inherited in an autosomally dominant fashion but with complete suppression of the phenotype after maternal transmission. This phenomenon is attributed to genomic imprinting and causes the disease to skip generations if transmitted along the female ancestral line. The PGL1 gene that is associated with maternally imprinted head and neck paragangliomas is located on the long arm of chromosome 11 and encodes for SDHD, a subunit of cytochrome b558 in the mitochondrial respiratory chain complex II.[18] A number of mutations in the SDHD gene have been isolated in head and neck paraganglioma families from the US, the UK, Australia and the Netherlands. A second locus (PGL2) also on chromosome 11, that has a similar maternal imprinting, has been linked to paragangliomas in a single family, but not yet isolated.[133;199] Recently a third gene (PGL3) that shows a normal autosomal dominant inheritance, has been isolated in a German family.[156] This gene encodes for SDHC a mitochondrial respiratory chain protein closely related to SDHD.[155] Paragangliomas are the first tumors that are related to a functional disturbance in the mitochondrial respiratory chain. Although the function of these proteins suggest a relation to the chemosensory function of the carotid body, the tumor biology of head and neck paragangliomas is still subject to speculation. Moreover, the epigenetic mechanism that leads to imprinting of the disease after maternal transmission of the PGL1 and PGL2 genes is likewise obscure.

Since PGL1 is isolated it is possible to identify hereditary cases of head and neck paragangliomas in the absence of affected family members. Recently we analyzed 55 of such isolated cases and found germ line mutations in SDHD 22 patients (40%).[200] Although the Netherlands may have an elevated number of hereditary cases, due to the fact that many of these patients have a common founder, the number of unrecognized hereditary cases was surprisingly high. As a consequence the total of ascertained hereditary cases in our hospital has risen to approximately 65%. Although other populations will have lower prevalences it is likely that the number of hereditary cases in these populations, usually quoted to be 10%, is underestimated as well. The underestimation of the prevalence of hereditary head and neck paragangliomas follows from the genetic epidemiology of the disease. Like with every other rare inherited disease the infliction of two or more family members proofs the hereditary nature of the disease, whereas a negative family history does not proof the contrary. The lower the chance that gene carriers develop symptoms

of their disease the more likely it is that a familial trait is overlooked. Head and neck paragangliomas are a good example since the disease is completely suppressed after maternal transmission and does not cause symptoms in 34% of the carriers of the paternally inherited SDHD mutation. (Chapter 4)
With the isolation of PGL genes it is now possible to test patients and their family. Absence of a SDHD or SDHC mutation however, does not exclude a mutation in PGL2, or other not yet identified genes. Moreover, it will be difficult if not impossible to obtain a large unbiased population of head and neck paraganglioma patients in order to determine the prevalence of hereditary head and neck paragangliomas. For this study we designed a simple statistical method to estimate the number of hereditary cases in a certain population, using the peculiarity of hereditary cases to appear multicentric.

Patients and Methods

Three study populations were defined. First we included all patients with head and neck paragangliomas treated in our hospital between 1960 and 1999. Second we used the data published by Elders (1962), who made an inventory of all patients with head and neck paragangliomas diagnosed in the Netherlands between 1945 and 1960.[53] Third we used the study of Lawson (1980) who comprehensively reviewed all published cases of head and neck paragangliomas at that time.[120]
We assumed that every patient who develops multiple paragangliomas is hereditary, regardless of the family history. The proportion of the hereditary cases who develop multiple tumors can be established. Thus by counting the number of patients with multiple paragangliomas in a certain population we identify a known proportion of the total of hereditary cases in this population.
Hence we established the frequency of multicentric paragangliomas (MPG) among ascertained hereditary cases ($p_{mpg\text{-}h}$) in each study population. The relative frequency of hereditary paraganglioma (p) then follows from Bayes theorema as a function of the relative frequency of MPG in hereditary cases, $p_{mpg\text{-}h}$, and the total relative frequency, p_{mpg}, of MPG observed: $p_{mpg}=p_{mpg\text{-}h}\ p$ and thus $p=p_{mpg}/p_{mpg\text{-}h}$. The confidence interval of p was calculated using the profile loglikelihood.

Results

The results are summarized in Table 8-1. The LUMC series of head and neck paraganglioma patients contained 183 patients with head and neck paragangliomas that were initially treated in our institution. In 107 cases the hereditary origin was ascertained. Multicentricity was observed in 53 (50%) of the hereditary cases. In the total population multiple paraganglioma were present in 71 patients. The estimated total of hereditary cases of head and neck paragangliomas in our series is $\frac{1}{50\%}\times\frac{71}{183}=78\%$ (95% confidence interval 66%-92%). Elders found 101 patients with head and neck paragangliomas. Thirteen were known to have a hereditary origin. The prevalence of multiple paragangliomas among these cases was 39%. The total number of patients with multiple paragangliomas was 19. The estimated total of hereditary cases in this population is 48 % (95% confidence interval 34 -70%).

Lawson found 2600 published cases of head and neck paraganglioma, including 304 patients (12%) with multiple paragangliomas. In series describing hereditary cases 30% of the patients had multiple tumors. The 304 patients found in this series therefore comprise approximately 30% of the total number of hereditary cases. The estimated prevalence of hereditary paragangliomas in this group is 39% (95% confidence interval 35-43%).

TABLE 8-1 Results

Series	Patients	Multicentricity	Muticentricity in Hereditary Patients	Estimated % hereditary (95% CI)
LUMC	183	71	50%	78% (66-92)
Elders	101	19	39%	48% (34-70)
Lawson	2600	304	30%	39% (35-43)

Discussion

Assuming that all cases with multiple paragangliomas are hereditary we have estimated the prevalence of hereditary head and neck paragangliomas in three populations. This assumption can be made because the low incidence of head and neck paragangliomas (<0.2 per 100,000 per year) reduces the chance of accidental development of two of these tumors almost to zero.[160] The patients who develop multiple paragangliomas must therefore have a predisposing factor, very likely an inherited gene defect. The only generalized disease that is assumed to lead to carotid body hyperplasia or carotid body tumors is sustained hypoxia.[87] However, the phenomenon of carotid body hyperplasia is exclusively seen in high altitude dwellers and the carotid body tumors observed in dwellers of the South American Andes are mostly unilateral and show a high female preponderance.[173]

Isolated cases with head and neck paragangliomas from the Netherlands had mutations in SDHD in 40%.[200] Absence of a SDHD mutation however, does not exclude a mutation in PGL2 or PGL3, or other not yet identified genes.

The study populations we defined were not uniform. First of all, the patients in the LUMC are biased towards a greater number of hereditary cases due to the research we perform in this field. We routinely perform high resolution MRI of the entire head and neck region to detect small paragangliomas without symptoms. Moreover the follow-up in this group is probably longer, which leads to a higher incidence of multiple tumors in both ascertained hereditary cases as isolated cases. Finally the LUMC is situated in a region in which most head and neck paraganglioma patients are related to a common founder which has probably led to a higher incidence of hereditary cases.[185] In the two other study populations modern imaging techniques were not available. The study of Lawson is a review cases from the international literature and could be regarded as a general population. However, it is possible that

cases with multiple paragangliomas are more likely to be published. At the other hand many series include only one paraganglioma site of origin and do not mention multicentricity, except bilateral occurrence.

The series of Elders is a meticulous description of all patients in the Netherlands diagnosed in a 25-year period. No special attention was given to hereditary cases. Hence it is possible that these figures better reflect the prevalence of hereditary cases in the Netherlands.

In all study populations the estimated prevalence hereditary cases of head and neck paragangliomas was much higher than the prevalence of ascertained familial cases. Depending on the population the estimated prevalence of hereditary head and neck paragangliomas ranges between 40 and 80%.

With this study we do not want to provide exact figures concerning the prevalence of hereditary head and neck paragangliomas. The presented data show that these figures will greatly depend on the studied population. Nonetheless it seems clear that familial paragangliomas are much more likely to occur than generally assumed. The much quoted 10% of hereditary cases is only based on the unreliable family history; the expected percentage will be above 35%. Every patient is therefore at risk to develop bilateral paragangliomas and hence bilateral cranial nerve palsies. A thorough family history including more than 2 generations is obligatory in the work up of head and neck paragangliomas, as is imaging of the entire head and neck region and long term follow up.

Chapter 9

Genetic Counseling in Hereditary Head and Neck Paragangliomas

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First Experiences with Genetic Counselling Based on Predictive DNA Diagnosis in Hereditary Glomus Tumours (Paragangliomas)
Oosterwijk JC, Jansen JC, Schothorst van EM, Oosterhof AW, Devilee P, Bakker E, Zoeteweij MW, vd Mey AGL. J Med Genet 33:379-383, 1996

Introduction

Tumor growth in the paraganglia tissue of the head and neck (for example, carotid bodies, jugular bodies, vagal bodies) is a rare disorder affecting ~1 in 100,000 people.[115] Pooled data from Dutch pathological laboratories show a mean incidence of 25 (operated) cases a year (1986-1992) which corresponds to approximately 0.26 in 100.000 (Source: PALGA foundation Amsterdam 1995). The Dutch National Cancer Registry registered 90 glomus tumors over a ten years period (Source: SIG foundation, Utrecht 1990).

Symptoms usually occur between 15 and 45 years of age and consist of local swelling and cranial nerve injury and are rarely associated with vasoactivity as in pheochromocytomas. The percentage in which malignant degeneration occurs is unclear but may be between 4 and 16%. Metastases are seen in less than 5% and multiple tumors in ~5%.[16;79;140;144] Although benign in nature, tumor growth is often progressive and can severely affect surrounding structures. This may lead to disfiguring local swellings and give rise to cranial nerve injury leading to facial asymmetry, deafness or hoarseness. In more severe cases the base of the skull can be involved. In general the therapy of choice is surgery, which is usually possible without major complications for small tumors. However, the safety of surgical intervention depends, among other things on the size and localization of the glomus tumor(s); vascular and neurological complications are frequent in more advanced cases. It is difficult however, to forecast tumor growth behavior and in many cases 'wait and see' could be an alternative approach, since life expectancy in affected patients is not significantly reduced.[138;142] Periodic screening of patients using MRI, CT scan or angiography, is the only way to detect early, symptom free growth of glomus bodies in order to be able to intervene at an early stage or to monitor the usually indolent growth pattern.[69]

In hereditary glomus tumors or paragangliomas (PGL) the growth of glomus bodies is predominantly in the head and neck region. PGL presumably forms a small fraction of all cases with glomus body tumors: according to published reports some 5 to 10% of cases are genetic in origin.[79] This figure may actually be substantially higher given the fact that often generations are often skipped and the clinical picture was not always recognized properly, as explained below. In familial cases multiple tumors, both synchronous and metachronous, occur in at least 30%.[79;142] Transmission of PGL is autosomal dominant with highly variable expressivity and reduced penetrance. The inheritance pattern has long been puzzling clinicians due to a very reduced penetrance.[8;10;14] Van der Mey et al.[143] postulated that the inheritance pattern is compatible with modification of gene expression by genomic imprinting. Only a paternally transmitted PGL gene leads to symptoms irrespective of the clinical status of the father, whereas a maternally derived PGL gene (irrespective of the clinical status of the mother) gives rise to carriership without symptoms. This explanation, known to occur in other neoplastic disorders,[59;117] was later supported by statistical analysis in Dutch families.[91;92] McCaffrey et al.[138] recently demonstrated genomic imprinting in a number of PGL pedigrees ascertained in the USA.

Hereditary PGL is relatively frequent in the surroundings of Leiden, the Netherlands, owing to a founder effect segregating in several relatively isolated villages.[185]

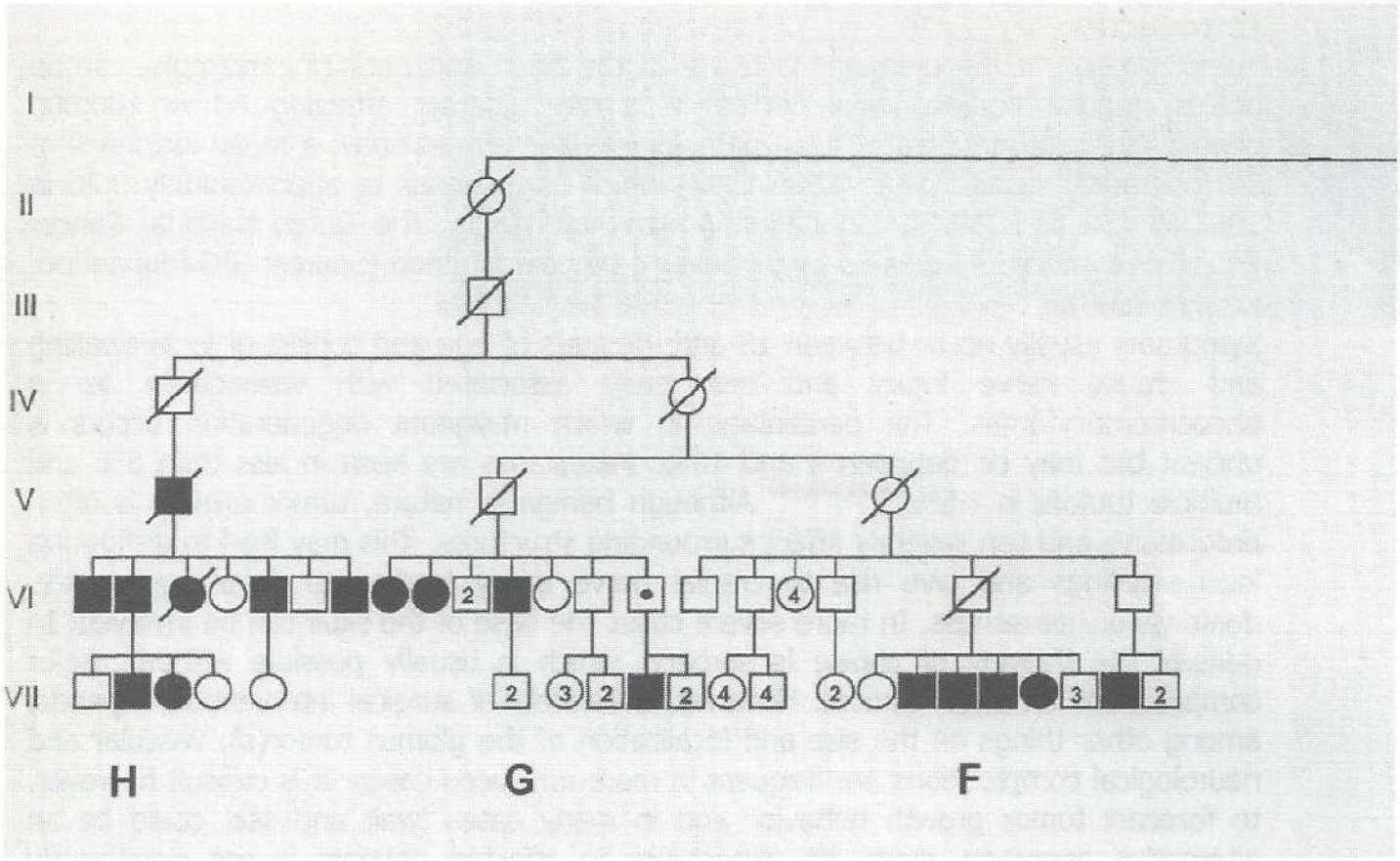
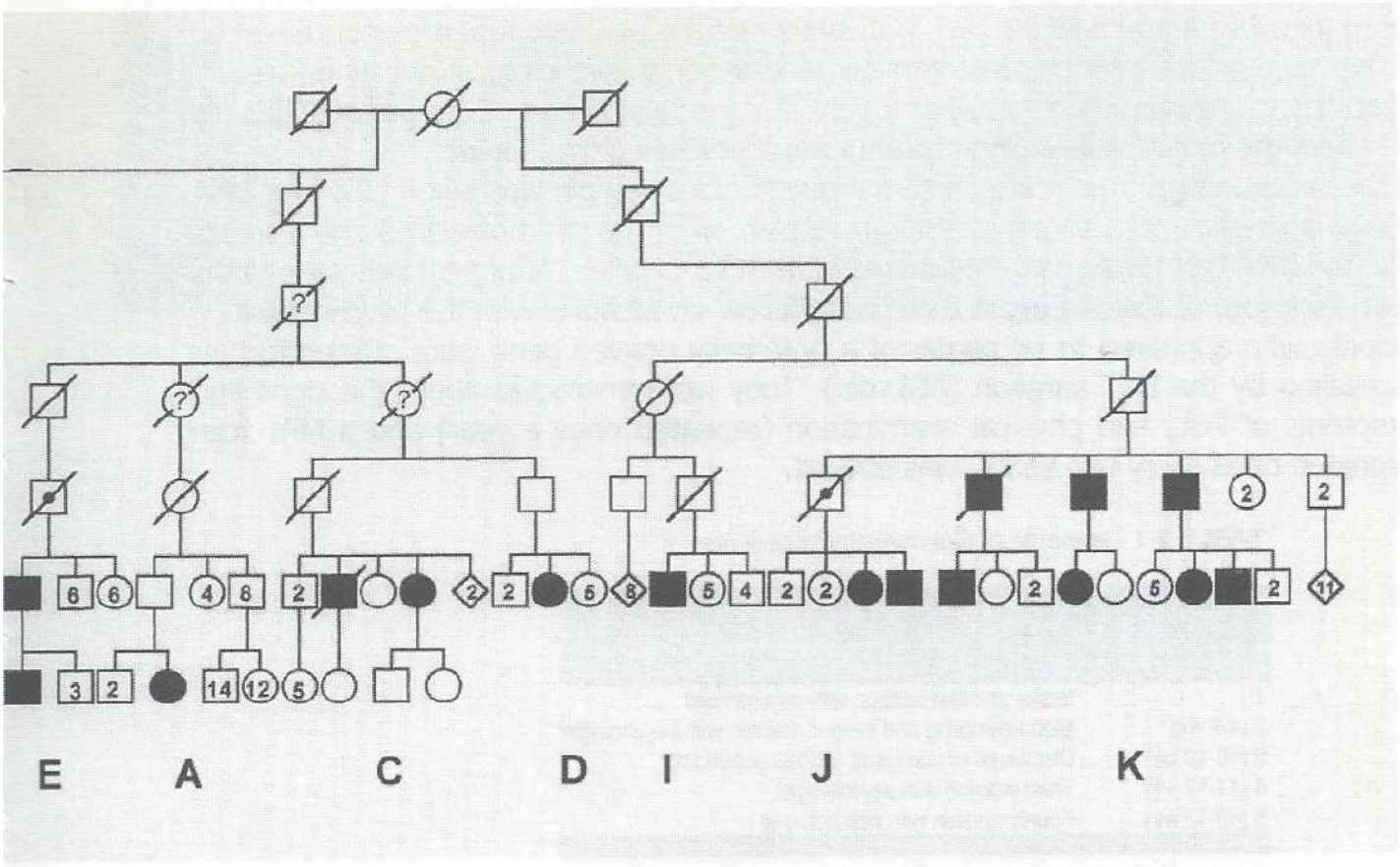


FIGURE 9-1
Extended Pedigree of the Leidem PGL family. Genealogical data lead to one common founder for several family branches. The pedigree is updated in 2000, and includes several newly diagnosed patients.

A gene for PGL has recently been mapped by linkage analysis to 11q22.3-q23.[91;92;184] This enables DNA diagnosis based on flanking markers in families where PGL is linked to this locus. Combining the correct interpretation of genomic imprinting with DNA diagnosis using flanking markers will greatly improve counseling accuracy for PGL. Given the implications of being at risk for PGL (periodic screening, multiple tumors, early surgical intervention) and given the genetic nature of the disorder, early establishment of the genotype may be useful both for clinical and genetic counseling. Moreover since predictive DNA testing may have significant psychological impact this aspect of counseling needs to be assessed. We report here of our experiences of genetic counseling in 32 persons of whom 20 were DNA tested according to a fixed protocol.

Patients and Methods

The patients are members of an extended Dutch pedigree in which linkage research was performed previously (Figure 9-1). Linkage to 11q22.3-q23 between markers STMY and CD3D was established firmly with a lod score of over 6.5 at $\Theta=0$.[91;92] as well as haplotype sharing for D11S938 to D11S908 in all patients.[184] Genetic counseling based on imprinting data and DNA linkage diagnosis using a fixed protocol was offered to the family members by means of a letter from the ENT Department. This letter was sent to



several key persons in the family who distributed the information further. Of the ~90 persons in this kindred that may have received the letter 68 members responded (written consent), of whom 32 (47%) opted for further counseling. The persons who opted for DNA testing were counseled according to a certain protocol and were asked to participate in a psychological study consisting of several questionnaires and sessions with a psychologist before and after DNA-testing. The design of the study was approved by the local ethical committee.

Counseling protocol

The counseling procedure was designed as a multi-disciplinary approach involving the Departments of Human – and Clinical genetics, and Otorhinolaryngology (Table 9-1).

At the intake the family histories were recorded and the clinical picture of PGL and the mode of inheritance was explained. Furthermore the procedure of DNA testing was explained and a prior risk was established using age dependant expression data of Jansen at al., based on the documented age at first symptoms in 77 familail cases known to the ENT Department. When possible we aimed at testing key persons high in the pedigree (for example a parent at risk) in order to avoid unnecessary testing (for example, in all children) and to prevent disclosure of unwanted information (for example, testing a 25% at risk person may disclose a parental haplotype).

Subsequently the psychologist (AO, MZ) explained the psychological protocol consisting of two pretest and two posttest sessions and several psychological questionnaires.[202] When the counselee objected to participate in the scientific part of the protocol this did not affect the routine counseling procedure and psychological support.
At the second appointment after 4 to 6 weeks a blood sample was taken (JCO) for DNA linkage analysis, and a second session took place with the psychologist. Some 8 weeks later the DNA test results were discussed with the counselee. Approximately six months after disclosure of the test result there were follow-up sessions with the psychologist.
Patients who appeared to be carrier of a paternally derived gene were offered further counseling by the ENT surgeon (AGLvdM). They were instructed about the signs and symptoms of PGL, and physical examination (repeated once a year) and a MRI scan (repeated once every two years) was offered.

TABLE 9-1 Timetable of Counseling Procedures

Session	Procedure
1	Intake and first session with psychologist
2 (4-6 wk)	Blood sampling and second session with psychologist
3 (10-12 wk)	Disclosure of test result (clinical geneticist)
4 (11-12 wk)	Third session with psychologist
5 (40-42 wk)	Fourth session with psychologist

Molecular analysis
DNA linkage analysis was performed using flanking polymorphic markers. From counselees who opted for presymptomatic DNA diagnosis or DNA carrier testing 20 ml EDTA blood was taken (JCO). In several cases also blood of the unaffected parent was taken (JCO) in order to establish the phase of the haplotypes. The blood was directly transported to the laboratory and all analyses were performed according to national diagnostic quality standards. The haplotype analysis was checked with the former research data of the extended pedigree whenever present (15 cases). Haplotype analysis was based on (from centromere to telomere) D11S527, D11S876, D11S927, D11S897, D11S560, D11S938, D11S1327, D11S1340, APOC3, D11S490, CD3D and D11S836.[1;82] More markers were used when the above mentioned were uninformative, e.g. D11S908, D11S939, D11S2082. The distance between the informative markers flanking the PGL region was between ~2 and 7 cM in all cases.[184]

Results
Sixty-eight people returned the form in which they could indicated whether they wanted further counseling or not. Thirty-six people refrained from further genetic counseling and DNA analysis. Five of these were clinically affected with PGL, 10 were potential carriers of a maternally derived allele, 4 were at risk of PGL (paternal allele) and of 17 their status was unknown.
Thirty-two (47%) persons opted for genetic counseling. Five patients were clinically

TABLE 9-2 Description of 20 Cases Where DNA Diagnosis was Performed Using Flanking Markers

Case	Sex	Age (y)	Prior risk (%)	Result	Follow-up ENT
Presymptomatic testing (paternal allele) (n=16)					
1-17	M	28	35	neg	
1-61	M	34	30	pos	scan +
1-62	M	33	30	neg	
1-63	M	26	35	pos	scan -
1-99	F	58	20	neg	
1-64	M	71	20	withdrew	
1-104	M	34	30	not informative	scan -
1-78	M	66	20	neg	
1-a	M	28	35	pos	scan +
1-113	F	24	40	neg	
1	M	23	40	neg	
1	M	18	45	neg	
9-47	F	33	30	neg	
8-40	M	45	25	neg	
8-15	F	39	25	pos	pending
8-16	F	37	30	neg	
Carrier testing (maternal allele) (n=4)					
1-34	F	61	50	neg	
1-40	F	64	50	pos	
1-117	F	43	50	neg	
9-88	M	70	50	neg	

affected and received extensive genetic counseling without the need for molecular diagnosis. The remaining 27 twenty persons were not affected as far as they knew; 20 were at risk for a paternal PGL allele and 7 for a maternal allele. Of these 27 cases, 23 were seen at the Department of Clinical Genetics, the remaining four cases learned their result in an indirect way because their parent at risk was among the persons tested and proved to not carry the PGL gene.

In the 23 symptom free cases seen, the closest affected relative according to family history was first degree in 18 cases, second degree in 3 cases and third degree 2 cases.

Deafness and facial palsy/asymmetry are the symptoms of PGL that counselees refer to as the clinical picture, but hoarseness, postsurgical mutilation, and death are also mentioned. The perception of the severity of PGL depended on the clinical picture of known affected relatives.
Of the 23 symptom free cases three counselees (two at disease risk, one potential carrier) refrained from further investigations after the intake session. In 20 symptom free cases DNA linkage diagnosis has been performed (Table 9-2). For all these cases a prior risk of carrying the PGL gene was determined (Table 9-2). For those at risk of a paternally derived gene we used the age at first symptoms curve as determined in the Leiden patient set by Jansen et al.
Of the 20 DNA diagnoses in 16 cases this concerned presymptomatic testing (paternal transmission). Four cases appeared to be gene carriers, ten are non-carriers, one DNA result is inconclusive owing to a recombination event and one person refrained from learning the test result. This man feared he could not cope with the feelings of guilt towards his children if he turned out to be a gene carrier. The four positive results increased the prior risk for in total 4 children to 50%, two potential maternal and two potential paternal transmissions. The eight negative results provided subsequently a result for over 11 children that were in a 25% at-risk situation that now changed to < 0.1%, that is the population risk of isolated PGL. This concerned a potential paternal transmission in 8 cases and a potential maternal transmission in 3 cases.
In 4 cases DNA diagnosis was performed for carrier testing (maternal transmission). In these cases their actual age does not affect the prior chance of carriership. One person appeared to be gene carrier, which increased the prior risk of 4 children to 50% of inheriting a maternal allele. Three cases were non-carriers, two female and one male. This made the risk of inheriting a maternal allele for 8 children and a paternal allele for 6 children negligible.
Four counselees, three who received a positive DNA result and the one case with an uninformative DNA result, visited the ENT out patients clinic for further counseling (Table 9-2). They were examined physically by the ENT surgeon and had a MRI investigation. Two out of three showed a tumor of the glomus caroticum (one of them was operated upon), one was negative on MRI. The case with the recombination had a normal MRI 5 years ago, and now showed no abnormalities on MRI.

Discussion

Paragangliomas is a relatively benign disorder leading to tumor growth in the head and neck region. According to published reports, at least 5% is hereditary; in our geographical region more than 50% appear familial and in these families genetic counseling should be offered. It is our experience that thorough family history data are needed before it can be concluded that a case is sporadic, because of the sometimes very mild symptoms in key relatives or the skipping of generations owing to genomic imprinting or both. This is illustrated by the fact that of our 20 people with a risk of 50% (at birth) of inheriting the PGL gene, two have a third degree and 3 have a second degree relative as the closest relative known to be clinically affected. Moreover as in many other well known genetic tumor susceptibility syndromes sporadic PGL cases with

multiple tumors are suspected to be due to an inherited mutation.[54]
Of the approximately 90 people in this pedigree that may have received our informing letter describing the possibility of DNA-based genetic counseling 68 people responded. Of these 32 (47%) opted for further counseling. Since the informing letter was forwarded by key persons in the pedigree we do not know the exact number of people that actually received this information. If all 90 were reached and non-responders are counted as negative responders than the uptake is 32 out of 90 (35%). If only 68 people were reached and all responded the uptake would be 47%. This means that in this family the uptake of genetic counseling is somewhere between 35 and 47%. This is still significantly higher than the uptake of 10-15% in Huntington's disease, a disorder where predictive testing offers no preventive or therapeutical consequences for the counselee. The percentage is lower however, than in familial adenomatous polyposis where it is some 90%, or in MEN2A (some 95%), where the preventive options are evident. Both disorders mentioned are potentially lethal in contrast to PGL, so the relative mildness of the disorder might also be a reason for the lower uptake.
The population that opted for counseling and testing did not significantly differ from those that responded negative with regard to age and gender (data not shown). In the first group 5 were affected with PGL, 20 were at risk of paternal transmission and 7 were at risk of maternal transmission. In the second group 5 were affected, 4 were at risk of a paternal allele, 10 were at risk of a maternal allele and in 17 cases the status was unknown. There seems to be a trend that being at risk to develop PGL (paternal transmission) is more often a motivation for testing than merely carriership.
DNA diagnosis in all counselees was performed against the background of extensive research linkage date of the same extended family. This made diagnostic linkage analysis relatively easy and excluded the problem of genetic heterogeneity. The latter is important since the issue whether there are two different loci for PGL on the long arm of chromosome 11 segregating in the Netherlands is still unresolved.[133;134] In our extended family linkage to 11q13 was excluded.[92;184] Of 20 DNA analyses performed only one case turned out to be uninformative due to a recombination event in the PGL region. In all other cases the DNA result had a certainty of over 99.9% since the chance of a double recombinant between the PGL locus and both flanking markers is very low.[184]
It is evident that genetic counseling and risk estimation are much more specific when based on parental origin of the PGL gene and on DNA diagnosis using flanking polymorphic markers. For people at risk of a paternal PGL gene the reliability of a DNA diagnosis performed once is age independent, less burdensome than (regular) MRI or angiography, and less expensive than most other procedures. DNA diagnosis is in fact the only way a person at risk of a maternal PGL gene can learn his or her status unless the disease already manifested itself in affected offspring. This DNA carrier diagnosis for an autosomal dominant disorder with no consequences for the 'patient's' health, even at young age, is unusual in clinical genetics. It will be interesting to compare the results of the psychological study with research findings about the detection of a BRCA1 or BRCA2 mutations in males. Data are scarce on the uptake and (psychological) implications in this category but feelings of (survivor) guilt might be anticipated.[52]

Four patients appeared to be carrier of a paternal allele at age 26, 28, 34, and 39 respectively. In two of them a glomus tumor was subsequently detected on MRI, and one has been operated upon recently. The fourth is awaiting MRI analysis. In all paternal gene carriers a MRI investigation is valuable in order to make comparison with subsequent imaging possible. Moreover, since glomus tumors can be removed more safely in an early stage, it is expected that regular MRI scanning on the basis of the demonstrated presence of a paternal PGL gene will improve patient management by the ENT surgeon.

The fact that 75% of DNA tests proved to be negative may be unexpected given an autosomal segregation ratio of 50%. This may be explained by the fact that five symptomatic counselees were excluded from DNA testing because their status was evident clinically. Moreover all persons tested for a paternal allele were adult (mean age 37 years) and so their prior risk was decreased (mean risk 28%) due to their age given the age related penetrance of PGL. The mean prior risk of 28% very well fits to the 25% positive test results.

The psychological impact of genetic counseling and predictive DNA testing for PGL has not been described as yet. It is unknown how people at risk cope with their test results. One might expect that the parental origin of the gene may affect psychological impact. Apart from comparison to other late-onset disorders a comparison with certain types of carriership would be interesting. Also the preventive and therapeutical aspects of PGL need to be taken into account when comparing counseling data to other late onset disorders. Preliminary data show that the main reason of DNA testing is for the sake of the children, and this applies especially for the older persons, whose risk to still develop clinically relevant glomus tumors is negligible. These and other psychological issues are currently being investigated and will be published in the future.

Conclusion

Since the genetic aspects and the clinical aspects (instruction, regular examination, treatment and prognosis) of PGL are rather complex and closely related we suggest that a multidisciplinary approach is desirable in counseling hereditary PGL. Our experiences to date show that genetic counseling gains significant accuracy when based on parent of origin, sex of the counselee and DNA linkage diagnosis. Moreover a normal DNA result may prevent unnecessary worries and investigations, whilst an established presymptomatic DNA diagnosis will guide clinical management. Further investigation into the natural history of PGL in gene carriers and into the psychological impact of DNA testing is desirable.

Chapter 7

Two Founder Mutations in the SDHD gene

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Nearly All Hereditary Paragangliomas in the Netherlands Are Caused by Two Founder Mutations in the SDHD Gene. Taschner P, Jansen JC, Baysal BE, Bosch A, Rosenberg EH, Bröcker-Vriends AHTJ, van der Mey AGL, van Ommen GJ, Cornelisse CJ, Devilee P. Genes, Chromosomes & Cancer, in press

In this final section the subsequent chapters of this thesis will be summarized and discussed with emphasis on the clinical consequences of the results. A summary in Dutch is presented in the next chapter.

Chapter 1. **The Paraganglion System & Head and Neck Paragangliomas**
This is a review of the paraganglion system and the tumors in the head and neck region that arise from it. The carotid body, a tiny structure residing in the bifurcation of the carotid artery, is the only paraganglion that has been extensively studied. It is a receptor organ sensitive for oxygen and carbon dioxide levels in the blood and it influences the breath rate. Although the other paraganglions in the head and neck, are not very closely related to the large arteries their microscopic appearance and nerve supply suggests a similar function.
The tumors arising from the paraganglia are called paragangliomas. These tumors are rare and usually benign. Although slow growing they can cause serious problems including invasion of the brain. By their close adherence to the large vessels and cranial nerves paragangliomas present a treatment dilemma. Sometimes the result of treatment is worse than the natural course of the disease.
Some new data are presented. Using the pooled data of the Dutch pathology labs (PALGA) we calculated the incidence of head and neck paragangliomas to be approximately 1 per 400.000 per year. The rate of malignancy, as defined by metastatic spread, was 1.4% in our series. Association with other diseases, frequently suggested by case histories, could not be confirmed. The paraganglioma of the adrenal gland (pheochromocytoma) was observed in ~2% of the cases.
Head and neck paragangliomas may be an inherited disease. The inheritance pattern is extraordinary and does not conform to the standard (Mendelian) laws of genetics. If the mutation is inherited from the mother the disease is completely imprinted and only reappears after a male descendant passes the mutation on to his children. Notably both sexes can be affected. The genetic mechanism of this mode of inheritance is still under investigation. One gene has been identified (PGL1, identical with SDHD, at chromosome 11q-23), one gene has been located (PGL2 at 11q13) and a third not imprinted gene (PGL3) has been identified as SDHC.[18;133;155]

Chapter 2. **Color Doppler Imaging of Paragangliomas in the Neck**
This chapter describes the use of a relatively new imaging technique. Ultrasound is a widely used method to assess tumors in the neck. It cannot discriminate paragangliomas from more common tumors like lymph node metastases. However, paragangliomas are much more vascularized than most other tumors in the neck. The change in wave length (Doppler shift) caused by the reflection of sound from moving objects (i.e. blood cells) makes it possible to depict blood flow. It is superimposed in color on the grayscale ultrasound image. With this technique we detected most paragangliomas in the study group. Strong reflections of ultrasound from the bony base of skull hindered the depiction of small paragangliomas arising from the nearby vagal body. In all paragangliomas we observed high blood flow, with a characteristically low resistance flow pattern. Although we did not use Color

Doppler to differentiate paragangliomas from other tumors in the neck, it seems to be a useful method. Its application is especially recommended for equivocal neck masses. Since multiple paragangliomas may occur simultaneously, magnetic resonance imaging is always required to screen the base of skull.

Chapter 3. **Growth Rate of Head and Neck Paragangliomas**

The management of head and neck paragangliomas is a balance between the risk of waiting and the risk of treatment. Most authors consider the risk of waiting to be to big for paragangliomas and are satisfied with the surgical results. This last opinion is remarkable since recent series of surgery for carotid body tumors have shown a peri-operative death rate up to 6%, cerebrovascular accidents in up to 20% and new cranial nerve palsies in up to 48% of the surgically treated cases.[15;111;126;149] This aggressive approach is based on a tradition in which every tumor should be radically removed. However, in order to improve the quality of life of the patients the natural course of the disease must compare unfavorable to surgery.

A clinical study among our patients showed little benefit of surgery of paragangliomas at the base of skull. We observed that many untreated patients showed little or no change in their complaints and grew old with the tumor in place.

For this chapter we studied one of the aspects of natural course resulting in the first report on growth rate of head and neck paragangliomas. For each patient we used two scans made with an average interval of 4.2 years. On each scan the volume was estimated assuming an egg-like shape of the tumor. One must be aware that this method includes many measurements and is not free from measurement errors. Based on other studies we regarded a volume increase of 20% a minimum to prove growth. Only 65% of the 48 tumors did increase that much. We calculated the time it would take each tumor to double in volume and observed a wide range –from 0.67 to 22.5 year- with a median of 4.2 year. Since malignant tumors usually have tumor doubling times <0.27 year, even the fastest growing tumor (tumor doubling time 0.67 year) was comparatively indolent.

Among the growing tumors we observed a tendency of decreasing growth rate with increasing size. This is common for most tumors. However, if the stationary paragangliomas were included, absence of growth was more often seen in small tumors than in tumors of intermediate size.

These results shed new light on the tumor biology of head and neck paragangliomas. For instance it contributes to an old hypothesis that paragangliomas first have a slow increase of the entire organ (hyperplasia) followed by a clonal outgrowth of transformed cells (neoplasia). Systematic–pre-treatment determination of tumor growth rate may provide more insight in the association between growth rate, tumor phenotype (histology and immunohistochemical expression patterns) and genetic defect. This eventually may enable the development of a multivariate prognostic model that can assist in the clinical decision making process for determining the optimal moment of treatment. With the current knowledge such a model would be very unsophisticated but by introducing every result on natural course, treatment and their impact on the quality of life of the patient, it would gradually improve.

Of direct clinical importance is the confirmation of the clinical observation that paragangliomas often remain unchanged for years. Aspects like direction of growth and encasement of nerves and vessels are very important in determining the symptoms of a tumor and -equally important- the outcome of surgery. Size however, is easy to assess and has shown to be of great importance in the natural course and surgical results of paragangliomas. If a 4% malignancy rate is accepted and the patient is willing to take the psychological burden of follow up with impending surgery, a 'wait and scan' policy can safely be applied on almost all head and neck paragangliomas. Hopefully this study increases the acceptance of this conservative management because informal communications learn that such a 'wait and scan' policy is applied more often than the published series suggest.

Chapter 4. **The Risk of Head and Neck Paragangliomas in SDHD Mutation Carriers**

Penetrance expresses the probability that a person with a germline mutation will get the corresponding disease. Head and neck paraganglioma is a disease of adults. Rare cases in newborns have been described, but generally the tumors are diagnosed in patients between 15 and 55 years of age. The penetrance of hereditary paragangliomas is therefore age dependant. The penetrance is practically zero until puberty and than gradually increases. Both genetic as well as tumor biological factors explain the late onset of the disease. Familial cases of head and neck paragangliomas have inherited a mutation from their father but also a normal gene from their mother. Experimental data suggest that it is only after the loss of the normal gene (wildtype allele) that paragangliomas will develop.[183] This secondary loss occurs more or less randomly usually during DNA replication, by chromosomal mechanisms like mitotic non-disjunction or mitotic recombination. The risk of such an event cumulates during life. If both genes are inactivated a paraganglioma will develop very slowly as we have seen in Chapter 3. It takes years before a palpable tumor is present and a delay of some more years between the onset of symptoms and the diagnosis is common.

Before the study presented here, one other estimation of the penetrance of this disease was published.[10] This study was performed in the Dutch family that later proved to be linked to another gene (PGL2).[133] In this family the penetrance was estimated to be 100% at the age of 45.

For the first time we were able to use genetic data to identify carriers of SDHD mutations. We found 48 persons at risk for paragangliomas. Twenty-four had symptoms but magnetic resonance imaging revealed 9 more patients, without symptoms. After accounting for age, the penetrance of paragangliomas was 100% at the age of 70 year. If only patients with symptoms were included the penetrance was much lower 66% at the age of 53 year. Thus from a genetic point of view the penetrance of head and neck paragangliomas is complete. The finding that only 66% of the carriers of a paternally inherited SDHD mutation experience symptoms of the disease is clinically much more important. It is especially important for the genetic counseling of carriers without symptoms. It means also that regular screening with

magnetic resonance imaging will not yield many early detected paragangliomas.
We must be aware that this study only includes one specific SDHD germline mutation and that other SDHD germline mutations, let alone germline mutations in other genes, might have other penetrance rates. Moreover the estimated percentage is based on the data from only a limited number of patients. However, it is certain that not all patients develop symptoms of paragangliomas, which confirms the growth rate study described in Chapter 3. With the increasing possibilities of genetic testing and counseling more data regarding the penetrance of SDHD and other genes will become available.

Chapter 5. **Confinement of PGL1 to a 2 cM Interval on 11q22-q23**
Before SDHD was identified as the major susceptibility gene for hereditary head and neck paragangliomas, PGL1, much work had to be done. First we located PGL1 on the long arm of chromosome 11 and then confined it to region 11q22.3-q23.[91;92] This region consists of approximately 16 million DNA-base pairs (16 cM) and therefore many possible locations for the pursued mutation. By then we had tested 6 families that would yield no extra information. Other families were too small to expect statistically strong data. However, we noticed that several families had ancestors that lived in the same region. Genealogical investigation led to the identification of a common ancestor for 11 branches of a large family. Hence we were certain that the descendants of this woman all carried the same mutation. We combined the genetic data of the patients, which showed that 9 branches were very similar. However, the remaining 2 branches had only a small chromosomal region that was identical with the other patients. Thus we reduced the putative PGL1 containing region from 16 cM to 2 cM (~2 million DNA-base pairs). Subsequent investigations, with newly isolated chromosomal markers revealed a second part on chromosome 11q23 that was identical in all branches. This region harbored the SDHD gene in which germline mutations could be discovered.[18]

Chapter 6. **Founder Effect in Hereditary Head and Neck Paraganglioma Families**
After we found a common ancestor for three families with head and neck paragangliomas (chapter 5) we expected that other families from this region would be related to this family as well. Therefore we performed a genealogical survey for 10 families from the province of *Zuid-Holland*. Nearly all ancestors of these families could be traced until ~1800 AD. Surprisingly none of them proved to be related.
However, comparison of the genetic data showed a very similar pattern in all families, statistically proving that they were all suffering from the same mutation, inherited from a common founder. This mutation has occurred at least 200 years ago. Given the geographical spread of the ancestors in 1800 AD, at least several generations could be added. Moreover, the fact that there were families of Roman Catholic faith as well as Reformed families suggests that the mutation has occurred before the Reformation that started in the Netherlands around 1560.
A founder effect explains the unusual high prevalence of hereditary cases in the

LUMC region (and presumably the whole of the Netherlands). At a clinical level this study once again confirms the relatively favorable natural course of head and neck paragangliomas, since a mutation will not be conserved for such a long period of time, if the concomitant disease reduces the reproductive fitness of the carrier.
We also may conclude that the variable clinical expression we observe in our patients is not caused by a different type of SDHD mutation. At the other hand, our findings cannot automatically be applied to other patient groups without a founder effect or patients that have other mutations in SDHD, PGL2 or PGL3.

Chapter 7. **Two Founder Mutations in the SDHD Gene**
After the SDHD gene was identified we searched for mutations in all families we had ascertained so far. We also included the cases that did not have a family history of paraganglioma. We confirmed that all families described in chapter 6 had the same mutation and subsequently showed that this specific mutation was present in 24 of the 32 Dutch families. Six families were related to another founder mutation and a single family had a thus far unique mutation in SDHD. As expected no SDHD mutation was found in the Dutch family that was previously linked to PGL2.
Analysis of 55 Dutch cases without a family history of paraganglioma showed inherited SDHD mutations in 22 (40%) of them. This confirms the hypothesis that the prevalence of hereditary head and neck paragangliomas is underestimated. Among the patients with multiple head and neck paragangliomas the prevalence of germ line mutations was 80%. We hypothesize that all patients with multiple tumors are hereditary cases. The few patients in this series without SDHD mutations then must be attributed to unrecognized mutations in SDHD, or mutations in other genes.
Three isolated cases from the UK all carried a founder mutation that was previously demonstrated in American families from English descent.
These results demonstrate that the founder effect of SDHD mutations is very strong. Studies in other populations may reveal concentrations of hereditary cases similar to that described in the Netherlands. PGL2, on the contrary, is only seen in a single family. Possibly PGL2 is a relatively recent mutation compared to PGL1. Nonetheless the pedigree published by van Baars et al. shows that PGL2 is at least 200 years old.[8]

Chapter 8. **The Prevalence of Hereditary Head and Neck Paragangliomas**
Tumors with a high incidence, such as colon cancer, can affect two sibs by chance. However, head and neck paragangliomas have a low incidence of ~0.26 per 100.000 per year and the coincidental occurrence of these tumors in two members of a family is extremely unlikely. In other words: If two family members develop paragangliomas it (almost) proves that the disease is inherited. On the other hand if a patient does not have a family history of head and neck paragangliomas it may still be a hereditary case for different reasons:
1. The patient may not know his family's medical history.
2. The family may have a very limited number of family members.
3. Previous generations may be imprinted due to repeated maternal transmission of

the mutation.
4. Other sibs may not carry the mutation.
5. Other sibs or even the father may not (yet) have developed a tumor.
6. Other sibs or even the father may not (yet) have developed symptoms of a tumor.
7. Other sibs or even the father may not (yet) have been diagnosed with a tumor.
8. Older generations may have had surgery for a paraganglioma but erroneously for another (histological) diagnosis.

This is why we hypothesize that the prevalence of hereditary head and neck paragangliomas is underestimated.
For the same reason that two family members will not be affected without a hereditary cause, it is very unlikely that an individual will develop two paragangliomas without having a germ line mutation. Therefor, our second hypothesis is that all cases with multiple paragangliomas are hereditary.
In Chapter 7 we have presented evidence for these hypotheses by showing a high prevalence of SDHD mutations among isolated cases, especially among those with multiple tumors.
If we know how many of the ascertained hereditary cases develop multiple paragangliomas we can estimate the total number of hereditary cases in a given population by counting the multicentric patients. With this method we estimated the prevalence of hereditary cases to ~78% among the LUMC-patients; ~48% among the Dutch patients diagnosed between 1945 and 1960; and ~39% among the patients with head and neck paragangliomas published until 1980.
This means that rather than a 10% prevalence of hereditary cases the real prevalence will be much higher in most populations. All studies that did not demonstrate a difference between hereditary cases and alleged sporadic cases must be revised. For example, it is peculiar that the age of onset between these two groups has not yet shown a considerable difference. Growth rate and histological criteria might differ as well. The Dutch population may not be very suitable for such a study as most cases are attributed to SDHD mutations. However, the variation of some of these parameters, found in our studies is large so that large groups must be included in these genotype/phenotype studies.

Chapter 9. **Genetic Counseling in Hereditary Paragangliomas**
Before the mutations in SDHD were detected, the approximate location of PGL1 in a large family yielded sufficiently accurate data to predict carriership. Our experiences with this family are described in this chapter. The response was relatively high. Most family members were not concerned about their own health, but were rather counseled with regard to their children or grandchildren. We had the impression that the experiences of close relatives with head and neck paragangliomas greatly influenced the attitude of the counselees towards the disease. Psychological problems with a favorable test result ('survivor guild'), as seen in syndromes like Huntinton's disease, were not observed.
The extraordinary inheritance pattern of hereditary head and neck paragangliomas is

difficult to comprehend for people unfamiliar with genetic counseling. The disease itself makes professional counseling even more wanted as testing is not unequivocally beneficial for the counselee.

1. The onset of the disease cannot be prevented with presymptomatic DNA testing.
2. If a diagnostic screening protocol is used a large number of scans will be made with little result given the late onset of the disease.
3. Not all head and neck paragangliomas require treatment. Unfortunately we can not yet predict tumor behavior. After presymptomatic diagnosis we probably will treat paragangliomas that would cause no harm in their natural course.
4. The confirmation of a hereditary disease, especially a tumor syndrome that is not well known by most physicians, may lead to rejection by insurance companies, mortgage brokers and employers.
5. Exclusion of a SDHD mutation does not exclude a hereditary cause since other genes are involved.

Active participation of the surgeon is needed in the genetic counseling of these patients in order to determine the clinical consequences of counseling. If, at the other hand, testing does not have clinical consequences for the patient, it still can have psychological consequences and influence the founding of a family. These aspects are familiar to the genetic counselor and psychological help can be offered. The legal aspects of counseling are first and foremost a political problem but should not be disregarded by the counselor. Now the mutations are known we cannot withhold DNA test to our patients and their families. However, before presymptomatic testing the surgeon and the counselor should point out the possible consequences (pre-test counseling). At this time, ascertained carriers of the paternally inherited mutation are offered radiological follow up, because in our opinion, early detection of paragangliomas offers the best opportunity to determine a treatment strategy.

Conclusions

At least 66% of the head and neck paragangliomas in the Netherlands are inherited. Nearly all inherited paragangliomas in the Netherlands are caused by two founder mutations in SDHD. If paternally inherited the SDHD Asp92Tyr mutation has a ~100% penetrance but not all patients will develop symptoms of head and neck paragangliomas. This implicates that every patient in the Netherlands should be treated as a hereditary case. Multiple tumors must be anticipated in the treatment strategy. Conservative management should be considered. Genetic counseling should be offered.

The estimations of growth rate confirm the indolent nature of these tumors. Growth could only be detected in a small majority of the studied cases. However, the natural course of head and neck paragangliomas remains unpredictable. Growth curves provided by a longer follow up may be of use. But the relation between growth and symptoms still has to be investigated. Further unraveling of the tumor biology could

also contribute to predict and maybe influence the natural course. Surgical results will continue to improve but with the advent of stereotactic radiotherapy this treatment modality will undoubtedly be strongly promoted by some. Large tumors however will not soon be eligible for irradiation. The value of both treatment modalities can only be evaluated properly with more knowledge of the natural course of the disease.

Because a large majority of our patients must be attributed to the same mutations it is not clear whether the results are applicable to patients with other inherited mutations or to true sporadic cases. The large variation in age of onset, growth rate, clinical presentation and histology found in Dutch patients suggest that statistical differences will not easily be found. Large studies in other (foreign) populations that are needed to compare the genetic status (genotype) to the clinical situation (phenotype) phenotype, are difficult to accomplish. Our statistical analysis however, suggests that also in other populations a higher incidence of hereditary cases must be expected.

The genetic counseling of patients with head and neck paragangliomas has specific genetic and psychosocial aspects. The recent identification of the not imprinted SDHC mutation complicates the counseling even more. The most troublesome aspect of genetic counseling in head and neck paraganglioma patients is the risk that insurance companies and employers, may deny these patients (even though they do not have a reduced life expectancy and may never experience symptoms of the disease). Pre-test counseling is therefore of great importance, especially of patients without a family history of paragangliomas, and should be provided by clinical geneticists in close cooperation with the surgeon.

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Samenvatting

Inleiding

Dit proefschrift gaat over een zeldzame tumor die in Nederland vaak glomustumor of chemodectoom wordt genoemd, maar waarvan de officiële naam paraganglioom is. Een aparte vorm van paragangliomen komt voor in het KNO gebied en wordt daarom hoofd-hals paraganglioom genoemd (engels: head and neck paraganglioma). Hoofd-hals paragangliomen kunnen erfelijk zijn en hebben meestal een zeer bijzondere overervingsvorm.
Het proefschrift is het resultaat van een vruchtbare samenwerking tussen de afdeling KNO van het LUMC en de afdelingen Humane en Klinische Genetica, Radiodiagnostiek en Pathologie. Dit wordt weerspiegeld in de onderwerpen die in dit proefschrift aan de orde komen. Centraal staan het natuurlijk beloop van de aandoening en verschillende erfelijke aspecten. In de algemene beschouwing worden de klinische consequenties van het onderzoek benadrukt.

Hoofdstuk 1
Het paraganglion-systeem en paragangliomen

Hier wordt eerst het complexe paraganglion-systeem waaruit paragangliomen ontstaan, besproken. In tegenstelling tot de hormoonafscheidende paraganglions die in de buik voorkomen (met als belangrijkste vertegenwoordiger het bijniermerg), zijn de paraganglions in het hoofd-halsgebied (in aanleg) receptoren die veranderingen in de zuurstofspanning waarnemen. De drie belangrijkste hoofd-hals paragangliomen ontstaan in de splitsing van de halsslagader, langs de 10^{e} hersenzenuw hoog in de hals en bij het middenoor.
Vervolgens worden in dit hoofdstuk de kenmerken van hoofd-hals-paragangliomen in algemene zin besproken. In de eerste plaats zijn ze zeldzaam. Wij schatten de incidentie in Nederland op 0,16 per 100.000 personen per jaar. Hoofd-hals-paragangliomen ontstaan meestal op volwassen leeftijd (gemiddeld 45 jaar), zowel bij mannen als bij vrouwen en in alle rassen. Soms zaait de tumor uit naar lymfeklieren of andere organen maar dit is gelukkig zeldzaam; bij onze patiënten in 1,4% van de gevallen. Meestal is een hoofd-hals paraganglioom dus goedaardig en groeit hij langzaam. De sterke doorbloeding en de nauwe relatie met bloedvaten en zenuwen maken het makkelijker om de diagnose te stellen (tegenwoordig met MRI-scan), maar bemoeilijken de chirurgische behandeling. Vroeger gingen mensen niet zelden dood aan de behandeling; nu komt het regelmatig voor dat hersenzenuwen die o.a. de stembanden laten bewegen opgeofferd worden. Hoe groter de tumor hoe slechter de resultaten van behandeling. Enerzijds is vroegtijdige operatie dus te verkiezen, anderzijds kan dit leiden tot ernstige klachten bij mensen die tevoren nauwelijks iets merkten en bij wie, gezien de langzame groei van de tumor, behandeling misschien nog lange tijd uitgesteld had kunnen worden. De studie naar de groeisnelheid is een van de onderwerpen van dit proefschrift en wordt beschreven in hoofdstuk 3.

Het andere zwaartepunt van de dissertatie is de erfelijkheid van hoofd-hals paragangliomen. Deze is bijzonder omdat de tumoren niet ontstaan als de erfelijke afwijking wordt doorgegeven door een vrouw; zij komen dus alleen van vaderszij. In tegenstelling tot geslachtsgebonden overerving kunnen de kinderen wel de aanleg voor hoofd-hals paragangliomen van hun moeder krijgen, maar zijn dan alleen drager van de ziekte. Een ander verschil met geslachtsgebonden overerving is dat (als ze het van hun vader krijgen) zowel mannen als vrouwen tumoren kunnen ontwikkelen. Eigenlijk lijkt de overerving dus normaal te verlopen maar wordt de ziekte onderdrukt (engels: imprinting) door de vrouwelijke overdracht. Hoe dit mechanisme van 'genomic imprinting' werkt is vooralsnog onderwerp van speculatie.

Hoofdstuk 2
Kleurendoppler-onderzoek van hoofd-hals paragangliomen
Echografie, onderzoek met ultrasoon geluid, wordt veel gebruikt als eerste onderzoek van tumoren in de hals. Met dit geluid kan ook stromend bloed worden waargenomen door meting van het Doppler effect, de veranderende toonhoogte bij een bewegende geluidsbron (hier het geluid dat terugkaatst van de stromende bloedcellen). De Doppler-informatie kan in kleur worden geprojecteerd in het zwart-witte conventionele echobeeld. Omdat paragangliomen zich vooral onderscheiden door hun sterke doorbloeding wordt de diagnose met de echo vergemakkelijkt als de bloedstroom met Doppler-techniek zichtbaar wordt gemaakt. Uit het onderzoek blijkt dat glomus vagale tumoren soms niet af te beelden zijn omdat ze te dicht bij het, niet met echo te onderzoeken, bot van de schedelbasis liggen. Palpabele glomus vagale tumoren en alle glomus caroticum tumoren zijn goed te zien en hebben een typische doorbloeding met hoge stroomsnelheden. Omdat echografie de schedelbasis, met daarin ook de paragangliomen bij het oor, niet kan afbeelden, is dit onderzoek niet geschikt als al wordt vermoed dat het een paraganglioom is, want dan moet het hele hoofd-hals gebied worden onderzocht. Het is wel zinvol om de kleurendoppler te gebruiken bij onderzoek van tumoren in de hals van onbekende oorsprong.

Hoofdstuk 3
Bepaling van de groeisnelheid van hoofd-hals paragangliomen
Bij een behandeling moet altijd worden afgewogen of de schadelijke effecten van die behandeling opwegen tegen het resultaat. Aangezien de behandeling van hoofd-hals paragangliomen ernstige gevolgen kan hebben is het belangrijk om te weten wat er gebeurt als je ze niet behandelt; het zogenaamde natuurlijk beloop. Hiervan is echter weinig bekend want veel hoofd-hals paragangliomen worden, in de meeste ziekenhuizen, spoedig na ontdekking geopereerd.
Een belangrijke factor in het natuurlijk beloop is de groeisnelheid. Aangezien in het LUMC eerder al was gekozen om in veel gevallen eerst af te wachten of een tumor groeit, door de patiënt klinisch en radiologisch te vervolgen, kon gebruik gemaakt worden van de scans om te berekenen hoe hard de tumoren groeiden. Aan de hand van opeenvolgende metingen werd de tumorverdubbelingstijd berekend. Het bleek

dat 35% van de 48 paragangliomen in het geheel niet groeide en dat de rest een verdubbelingstijd had, variërend van 0,67 tot 22,5 jaar (mediaan 4,2 jaar). Opmerkelijk genoeg waren het vooral de hele kleine tumoren én de hele grote tumoren die niet groeiden. Was er wel sprake van progressie dan werd dit minder naarmate de tumor groter was.
De belangrijkste conclusie van dit alles is dat het verantwoord lijkt om even af te wachten voordat een patiënt geopereerd wordt. Omdat de ingreep risico's met zich meebrengt is de beslissing om daartoe over te gaan makkelijker als met MRI is aangetoond dat er sprake is van groei.

Hoofdstuk 4
De penetrantie van PGL1
De penetrantie van een gen drukt de kans uit dat de eigenschap waarvoor dat gen codeert ook tot uiting komt. Bij een gemuteerd gen betekent het dus de kans dat je als je de mutatie hebt, ook daadwerkelijk ziek wordt. Uit het voorgaande is al gebleken dat er iets vreemds is met de penetrantie van hoofd-hals paragangliomen omdat gendragers die de mutatie van hun moeder erven de ziekte nooit krijgen (een penetrantie van 0). Omdat hoofd-hals paragangliomen doorgaans op volwassen leeftijd tot uiting komen, geldt voor degenen die de mutatie van hun vader krijgen dat de penetrantie leeftijdsafhankelijk is. Dit komt waarschijnlijk doordat er in de loop van het leven eerst een tweede genetische verandering moet ontstaan voordat er een tumor gaat groeien. Daarnaast groeien de tumoren zo langzaam (zie hoofdstuk 3) dat het nog jaren duurt voordat een tumor ook daadwerkelijk klachten veroorzaakt.
Voor de erfelijkheidsadvisering is het van groot belang dat de penetrantie van een ziekte bekend is. Het kan voorkomen dat iemand weliswaar de erfelijke aanleg heeft voor een ziekte, maar dat de kans dat hij of zij daadwerkelijk ziek wordt erg klein is.
Bij de studie die in dit hoofdstuk wordt beschreven is gebruik gemaakt van moleculair genetische gegevens, waaraan kon worden gezien wie in de bestudeerde familie de mutatie had en van de klinische gegevens, waaruit bleek wie er was aangedaan. Als gekeken werd naar het ontstaan van symptomen, bleek dat de penetrantie op de leeftijd van 53 jaar maximaal was en dat de penetrantie dan slechts 66% bedroeg. Als echter de gegevens werden gebruikt van de symptoomloze dragers van de mutatie die toch een MRI-scan hadden ondergaan, bleken daarop weldegelijk kleine tumoren zichtbaar te zijn en steeg de penetrantie naar 100% bij de leeftijd van 70 jaar.
Dit betekent dat dragers van de langs vaderszijde verkregen mutatie weliswaar een grote kans hebben om in de loop van hun leven een paraganglioom te ontwikkelen maar dat dit lang niet altijd tot klachten hoeft te leiden.

Hoofdstuk 5
Nadere localisatie van het PGL1 gen
In dit hoofdstuk wordt een studie naar de localisatie van het tot dan toe onbekende PGL1 gen beschreven dat verantwoordelijk is voor erfelijke paragangliomen in het

hoofd-hals gebied. Ondertussen is bekend dat dit gen codeert voor SDHD, een enzym dat bijdraagt aan de energievoorziening van de cel. Dit gen is gevonden door steeds nauwkeuriger te onderzoeken welk gedeelte van het erfelijk materiaal steeds identiek bleef bij de patiënten in een familie en niet aanwezig was bij gezonde personen. Dit leidde eerst naar het 11^{e} chromosoom en daarna tot een steeds kleiner gebied op dat chromosoom. Tenslotte werd een aantal genen in het afgegrensde gebied, die op theoretische gronden kandidaat waren om hoofd-hals paragangliomen te veroorzaken, nauwkeurig bekeken, waarbij in het SDHD-gen een mutatie bleek te bestaan, die er voor zorgt dat het enzym niet goed werkt.
Het hier beschreven onderzoek werd gedaan in de fase waarin het gebied op chromosoom 11 werd beperkt. Dit gebeurde door het erfelijk materiaal van patiënten binnen een familie te vergelijken, in de hoop dat er maar een klein gebiedje bij allen hetzelfde was. De kans dat er in zo'n geval verschillen te vinden zijn is groter als de patiënten verre familieleden van elkaar zijn. Bij de eerst onderzochte takken van de familie viel dit tegen. Door stamboomonderzoek kon er echter een aantal nieuwe takken aan de stamboom worden toegevoegd, waarbij voor sommige familieleden de enige verwant een in 1776 geboren vrouw was. Het gebied van chromosoom 11, waarop zich het gezochte gen moest bevinden, verschilde in deze takken net voldoende om een aanzienlijke beperking van het doelgebied te bewerkstelligen.

Hoofdstuk 6
Een gemeenschappelijke voorouder bij hoofd-hals paraganglioom families
In hoofdstuk 5 werd beschreven hoe het door stamboomonderzoek mogelijk bleek om een aantal families uit de Leidse regio, die ogenschijnlijk geen familie waren, toch tot één voorouder te herleiden. Erfelijkheidsonderzoek bij andere families uit Zuid-Holland liet zien dat deze zeker ook aan elkaar verwant moeten zijn. Helaas leverde uitgebreid genealogisch onderzoek, waarbij de voorouders van deze families tot het jaar 1800 of daarvoor werden teruggezocht, geen nieuwe verwantschappen op. Dit betekent dat er sprake is van een reeds lang bestaande mutatie die langdurig in de gemeenschap blijft bestaan en kennelijk geen aanleiding geeft tot een verminderde voortplanting. Omdat de familie generaties lang in dezelfde regio is blijven wonen, heeft dit waarschijnlijk geleid tot toename van het aantal erfelijke gevallen, die allemaal berusten op dezelfde mutatie.

Hoofdstuk 7
SDHD mutaties in Nederlandse en buitenlandse patiënten
Bij de identificatie van het SDHD gen werden in Amerikaanse en Nederlandse families een aantal verschillende mutaties gevonden, die tot hoofd-hals paragangliomen leidden. Vervolgens hebben wij alle bij ons bekende Nederlandse families getest alsook 55 patiënten die geen paragangliomen in de familie hadden. Daarnaast werden een aantal buitenlandse patiënten onderzocht.
Er werden 3 mutaties gevonden in 32 Nederlandse families. De meest voorkomende mutatie in 24 families, een andere mutatie in 6 families en 1 familie met de derde mutatie. Slechts in 1 familie werd geen SDHD mutatie gevonden. In deze familie die

is geanalyseerd door de Nijmeegse kliniek blijkt een ander nog onbekend gen een rol te spelen. In twee Noord Amerikaanse en een Belgische familie werden nieuwe genetische afwijkingen gevonden.
Opvallend genoeg bleek 40% van de Nederlandse patiënten die geen familie met paragangliomen had, toch een erfelijke mutatie te hebben, waaronder 2 tot dan toe onbekende mutaties. Van de patiënten met meer dan één tumor had zelfs 80% zo'n mutatie. In 3 Engelse patiënten werd eveneens een mutatie aangetroffen die eerder in Amerikanen van Engelse afkomst werd aangetoond.
Deze resultaten bevestigen dat erfelijke hoofd-hals paragangliomen vooral ontstaan uit enkele oude voorvaderlijke mutaties. Het vermoeden dat veel mensen niet weten dat het in hun geval een erfelijke ziekte betreft (zie hoofdstuk 9), wordt bevestigd. Tevens laat het onderzoek zien dat mensen met meerdere hoofd-hals paragangliomen een zeer grote kans hebben om de erfelijke vorm van de ziekte te dragen.

Hoofdstuk 8
Hoofd-hals paragangliomen zijn vaker erfelijk dan wordt gedacht
In de literatuur wordt meestal gesteld dat ongeveer 10% van de hoofd-hals paragangliomen familiair is. Er zijn echter vele redenen om aan te nemen dat dit een onderschatting is. Het belangrijkst hiervan is dat het overervingspatroon en de penetrantie (hoofdstuk 4) zodanig is dat er soms geen andere directe familieleden zijn aangedaan en de familieanamnese dus ten onrechte negatief is. In hoofdstuk 7 werd hiervoor al bewijs gevonden.
In dit hoofdstuk wordt een eenvoudige statistische methode beschreven die het mogelijk maakt om in een willekeurige populatie patiënten met hoofd-hals paragangliomen het aantal erfelijke gevallen te schatten. De methode is gebaseerd op de hypothese dat alle gevallen met multipele tumoren erfelijk zijn. Dit op theoretische gronden; het is namelijk erg onwaarschijnlijk om door toeval twee heel zeldzame tumoren te ontwikkelen en de enige gegeneraliseerde oorzaak die we kennen is erfelijkheid. In hoofdstuk 7 wordt dit ook proefondervindelijk bevestigd.
Nu hebben we de methode toegepast op 3 populaties, namelijk patiënten uit het LUMC, een compleet overzicht van alle Nederlandse patiënten tussen 1945 en 1960, destijds verzameld door de Groningse chirurg Elders en tenslotte alle gepubliceerde gevallen bijeengebracht door Lawson in 1980.
Met deze methode schatten we dat in onze populatie het aantal erfelijke gevallen 78% bedraagt. Bij de Nederlandse populatie zou dit 48% zijn en wereldwijd 39%.
Hoewel dit maar schattingen zijn kan worden gesteld dat erfelijke hoofd-hals paragangliomen helemaal niet zo zeldzaam zijn als wordt gedacht en dat er dus bij elke patiënt rekening mee moet worden gehouden.

Hoofdstuk 9
Eerste resultaten van erfelijkheidsadvisering
Als er een erfelijke aandoening in de familie voorkomt kunnen er vragen rijzen wat dit voor gevolgen voor een individu en zijn of haar kinderen heeft. De ingewikkelde

overervingsvorm van hoofd-hals paragangliomen maakte professionele uitleg reeds gewenst. Daarna werd het mogelijk om bij niet aangedane familieleden de aanwezigheid van de mutatie aan te tonen of uit te sluiten waardoor het erfelijkheidsadvies nog complexer werd. Als bij een gezond iemand wordt aangetoond dat hij/zij de mutatie van zijn vader heeft gekregen en dus het risico loopt op paragangliomen, kan de ziekte niet worden voorkomen. Wel adviseren wij om eens in de paar jaar een MRI-scan te laten maken omdat we denken dat beter over eventuele behandeling kan worden beslist als de tumoren vroeg worden ontdekt. Men kan zich echter voorstellen dat dit stress met zich meebrengt en aangezien het ook nog zo is dat een heel aantal dragers nooit symptomen zal ontwikkelen (hoofdstuk 4), willen sommige mensen de uitslag van het onderzoek liever niet weten. Bij het hier beschreven onderzoek bleek dat de meeste leden van de onderzochte familie wel advies wilden, vooral met het oog op hun kinderen.
Tegenwoordig is het zelfs mogelijk om bij willekeurige patiënten te zoeken naar SDHD mutaties. Echter, als deze niet worden gevonden kan er nog steeds sprake zijn van een erfelijk geval, veroorzaakt door een ander gen. Dus aantonen van erfelijkheid kan, uitsluiten niet.

Conclusies

In dit proefschrift wordt een aantal nieuwe bevindingen gepresenteerd die het vaak indolente natuurlijk beloop en de unieke genetica van hoofd-hals paragangliomen bevestigen. Omdat het overgrote merendeel van de onderzochte tumoren veroorzaakt wordt door een SDHD mutatie verdient soortgelijk onderzoek bij andere patiënten populaties aanbeveling.
Men moet zich realiseren dat een onverwacht groot aantal patiënten de erfelijke vorm van de ziekte heeft, hetgeen het afnemen van een uitgebreide familiegeschiedenis rechtvaardigt, desgewenst gepaard met erfelijkheidsadvisering.
Daarnaast moet men zich voor het instellen van een therapie realiseren dat het natuurlijk beloop van de ziekte soms gunstiger is dan de uitkomst van de behandeling en een terughoudend beleid derhalve moet worden overwogen.

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Curriculum vitae

Jeroen Casper Jansen werd geboren op 10 juli 1964 in het AZL te Leiden. Na het voortgezet onderwijs aan de Vrije School te Den Haag, dat in 1983 werd afgesloten met het staatsexamen VWO, ging hij in Leiden geneeskunde studeren. Aan het eind van de doctoraalfase werkte hij, onder leiding van dr. M.D. Ferrari bij de afdeling Neurologie van het AZL mee aan een tweetal klinische studies met een anti-migraine middel. De co-schappen werden afgesloten met de kleurendoppler-studie die de aanzet vormde tot dit proefschrift. Na het artsexamen in 1992 was hij als dienstplichtig bataljonsarts gelegerd in Garderen bij 108 Verbindingsbataljon. Hierna was hij werkzaam als assistent op de afdeling KNO van het AZL (hoofd: prof. dr. J.J. Grote), aanvankelijk als AGNIO, later als assistent in opleiding. Een gedeelte van de opleiding werd gevolgd in het Rijnland Ziekenhuis, locatie St. Elisabeth te Leiderdorp (opleider: dr. J. Hulshof). Per 1 juli 1999 werd hij geregistreerd als KNO-arts. Tot 1 maart 2000 was hij werkzaam op de afdelingen KNO van het LUMC en AZR/Dijkzigt waarna hij een 2 jarig fellowship 'Oncologie van de Schedelbasis' begon, gefinancierd door het Koningin Wilhelmina Fonds. Het eerste jaar hiervan bestond uit een stage hoofd-hals chirurgie in het AZR/Dijkzigt en AZR/Daniel (hoofd: dr. P.P.M. Knegt). Momenteel werkt hij in het LUMC op de afdeling Neurochirurgie (hoofd: prof. dr. R.W.T.M. Thomeer). In juli 2001 zal het fellowship worden voortgezet aan de schedelbasischirurgie afdeling van het St. Vincent's Hospital in Sydney, Australië (hoofd: P. Fagan).

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Stellingen

behorende bij het proefschrift:

Paragangliomas of the Head and Neck

1. In Nederland moet een paraganglioom in het hoofd-halsgebied als familiair worden beschouwd, tot het tegendeel bewezen is.

2. Het aantonen van groei bij hoofd-hals paragangliomen kan niet het enige argument zijn om tot operatie te besluiten.

3. Presymptomatisch MRI-onderzoek mag misschien leiden tot het éérder behandelen van erfelijke paragangliomen, maar niet tot het behandelen van méér van deze tumoren.

4. De Asp92Tyr "foundermutatie" in PGL1 is voor de Reformatie ontstaan.

5. Dat Green et al. beweren glomus jugulare tumoren met minimale morbiditeit ("minimal morbidity") te opereren, wil niet zeggen dat hun operatieresultaten bevredigend zijn. (Laryngoscope 1994 104:917-21)

6. Het reconstrueren van een accidenteel of bewust onderbroken n. accessorius, is de moeite waard. (Arch Otolaryngol Head Neck Surg 1998 124:377-80)

7. "Graft versus host disease" ten gevolge van microchimerisme door foetomaternale celoverdracht, is een alternatieve oorzaak voor tot op heden aan auto-immuun reacties toegeschreven aandoeningen bij vrouwen. (Immunol Today 2000 21:116-8)

8. Gezien de effectiviteit van dubbelblinde behandeling met placebo, zoals die door zowel alternatieve als reguliere artsen veelvuldig wordt toegepast, is het onverstandig deze praktijk zondermeer af te wijzen.

9. Een kwaliteitssysteem borgt kwaliteit net zo min als een spelsysteem voetbalt.

10. Ten onrechte laten bedrijven die hun middelen aanprijzen met "dermatologisch getest", de uitslag niet zien.

11. Gelet op zijn/haar maatschappelijk belang zou een leerkracht op de basisschool tenminste het salaris van een hoogleraar moeten verdienen.

12. Als de dinosauriërs klimaatverandering destijds effectief hadden bestreden, dan waren er nu geen mensen, die zich over dit verschijnsel zorgen konden maken.

Jeroen Jansen Leiden, 17 Mei 2001

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