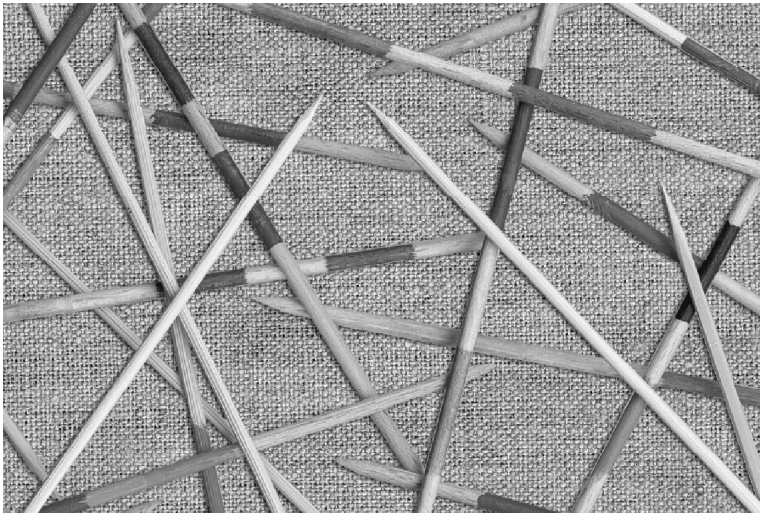


Summary



Summary

Chapter 1, objectives and general introduction

Chapter 1 is the introduction to this thesis and describes the main objectives and methods. It provides an overview of the history of VHL disease, as well as the clinical and molecular genetic aspects of the disease. In addition, the VHL protein and its possible functions are reviewed.

Clinical picture

Von Hippel-Lindau (VHL) disease is an autosomal, dominant inherited tumour syndrome. A germline mutation in the VHL gene predisposes carriers to tumours in multiple organs. These tumours may include haemangioblastoma in the retina and central nervous system (CNS), renal cell carcinoma, pheochromocytoma, islet cell tumours of the pancreas, and endolymphatic sac tumours (ELST), as well as cysts and cystadenoma in the kidney, pancreas, epididymis and broad ligament. The estimated prevalence of the disease varies between 1:31,000 and 1:53,000 persons. The disease is named after the German ophthalmologist Eugen von Hippel, who described retinal haemangioblastoma in 1904, and the Swedish pathologist Arvid Lindau who associated retinal and CNS haemangioblastoma with cysts of the kidneys, pancreas and epididymis in 1926. Most tumours in VHL patients are multicentric or bilateral, and manifest at a younger age than in patients without a VHL germline mutation. The mutation spectrum is heterogeneous, with mutations scattered throughout most of the VHL gene. Although some recurrent mutations have been reported, most families have their own unique germline mutation.

Penetrance of VHL disease is high, most carriers of a VHL germline mutation develop one or more tumours by the age of 60 years. The most common symptoms include: loss of vision, raised intracranial pressure, neurological deficits, paroxysmal raised blood pressure and local pain. The median expected survival, based on life table analysis, has been estimated at 49 years. At present, metastases from renal cell carcinoma and neurological complications from cerebellar haemangioblastoma are the most common causes of death in VHL disease. However, it is anticipated that intensive radiological and clinical monitoring, and advanced operation techniques will reduce both morbidity and mortality.

Objectives of this thesis

The main objective of this thesis is to identify patients and families with VHL disease by molecular genetic analysis. New families with VHL disease can be found by screening patients with VHL-like tumours (with a positive or a negative family history) for germline mutations. Identification of a VHL germline mutation confirms the clinical diagnosis. Presymptomatic DNA analysis and identification of carriers of VHL germline mutations in families then permits tumour development to be followed from a relative early age, and optimises the time at which treatment is carried out. Since clinical surveillance can be specifically directed towards carriers of a VHL germline mutation, the cost-effectiveness of annual monitoring is expected to improve. Moreover, tested individuals are no longer uncertain regarding their risk for developing the disease and family members who are non-carriers are relieved of the burden of repeated clinical monitoring.

Patients and methods

In order to detect new VHL families and patients, we took several initiatives to increase the number of persons possibly affected with VHL disease being referred for DNA diagnosis. Firstly, we asked the Departments of Ophthalmology in the university hospitals for patients with retinal haemangioblastoma. Secondly, we put an appeal in the journal of the Dutch Association for Neurology asking for patients with haemangioblastoma in the central nervous system. Thirdly, we approached approximately 80 medical specialist (including clinical geneticists, internists, endocrinologists, urologists, surgeons, neurosurgeons and paediatricians) with a known interest in hereditary tumour syndromes. Fourthly, we contacted the eight genetic centres in the Netherlands and fifthly, we distributed patient information via the Dutch VHL support group and the Internet.

DNA analysis for VHL in the Netherlands is performed at the Department of Medical Genetics, UMC Utrecht and in the Department of Clinical Genetics, Rotterdam University Hospital. DNA analysis included sequencing of the coding region and quantitative Southern blot analysis, complemented by Fluorescence in situ hybridisation (FISH) analysis when necessary. Clinical data were collected after patients had signed an informed consent form.

Summary of objectives

1. To detect VHL families and determine the family-specific germline mutation.
2. To identify presymptomatic relatives who carry a VHL germline mutation.
3. To screen for VHL germline mutations in patients with a single VHL-related tumour and without a distinct family history.
4. To improve DNA analysis techniques in identifying germline mutations in families where no mutation could be detected.
5. To collect clinical and genetic data to identify possible genotype-phenotype correlations.
6. To formulate national guidelines for diagnosis and periodic monitoring of VHL patients.

Chapter 2, clinical investigations

This chapter focuses on clinical aspects of VHL disease and describes radiological techniques (2.1) and guidelines for diagnosis and monitoring of the disease (2.4). Two organs that are involved in VHL disease are discussed in more detail, the kidney (2.2) and the eye (2.3).

Imaging of renal-, adrenal- and pancreatic masses

Section 2.1 reviews developments in the imaging of renal, adrenal and pancreatic masses in VHL disease. The imaging of other organs involved in VHL disease is described in chapter 1. Radiological imaging may favour early detection and monitoring of VHL-related lesions and is likely to lead to a reduction of morbidity and early mortality. Ongoing follow-up by careful radiological monitoring with ultrasound and especially MRI (magnetic resonance imaging) plays a central role in managing the disease.

We advocate annual monitoring of VHL lesions with MRI and ultrasound rather than CT (Computed Tomography). Ionising X-rays emitted by CT may mutate the DNA of the second (non-mutated) VHL allele. It is prudent to regard VHL as a disease where carriers of a VHL germline mutation have an increased risk for tumours, since tumourigenesis may be initiated with only one somatic hit.

Management of renal cell carcinoma

Since renal cell carcinoma occur often multiply and bilateral in carriers of a VHL germline mutation, a choice has to be made between careful radiological monitoring, nephron-sparing surgery and nephrectomy. This decision depends on size, growth and biological behaviour of renal tumours.

Renal cell carcinoma in our patients showed a slow growth rate (on average 0.3 cm/year) and asymptomatic patients presented with tumours of low-grade malignancy. In all patients, a fibrous pseudocapsule surrounded tumours. In five of 17 tumours, pseudocapsular invasion was observed and three of the five tumours had broken through the pseudocapsule. These patients did not show a less favourable outcome than those without pseudocapsular involvement by tumour growth. Multicentricity of renal cell carcinoma was relatively low (4.6 lesions per kidney). In two of the three patients that underwent a nephrectomy, only a single satellite lesion, in the direct vicinity of a renal cell carcinoma, was found in one kidney. Six tumours (1.8-5.5 cm) were enucleated by nephron-sparing surgery. During a mean follow-up of 30 months, renal function in these patients was well preserved. We concluded that, in our patients, renal cell carcinoma grew slowly, were of low grade, had a dense fibrous pseudocapsule and were hence good candidates for nephron-sparing surgery.

Ocular haemangioblastoma

Haemangioblastoma are the most common and early occurring tumours in VHL disease. In the eye the typical lesion is the peripheral retinal haemangioblastoma. Most ocular haemangioblastoma occur peripherally and 8% occur on the optical disc.

We describe long-term follow-up, ophthalmological data from 20 patients from six families, with special attention to the natural course of ocular haemangioblastoma. Five stages of the natural course of development of ocular haemangioblastoma are discerned and illustrated by fluorescein angiographic pictures. The patient from family C is an example of the ability of DNA analysis to find cases of VHL disease with a negative family history. After five years of extensive clinical monitoring, retinal haemangioblastoma are still the only manifestations of VHL disease. We advocate that only early detection and treatment of peripheral retinal haemangioblastoma can be expected to decrease the percentage of patients with impaired visual acuity. Since ocular haemangioblastoma are early tumours in carriers of a VHL germline mutation, ophthalmological monitoring (and subsequent treatment) of VHL patients and persons at risk should start as early as possible.

Guidelines for diagnosis and monitoring

The Dutch VHL Working group presents guidelines to enhance the early detection and treatment of VHL patients in the Netherlands.

Summary

For diagnosing VHL disease in a patient, both clinical manifestations and family history are important. Typical tumours that are associated with VHL disease are: retinal and CNS haemangioblastoma, pheochromocytoma, renal cell carcinoma, ELST and multiple pancreatic cysts. Multiple pancreatic cysts are specific for VHL disease because they are rare in the normal population. In contrast, renal or epididymal cysts occur more often in the normal population. In the presence of a positive family history, VHL disease can be diagnosed in a patient with a typical VHL tumour. In the absence of a VHL family history, two or more haemangioblastoma, or a haemangioblastoma combined with a further typical VHL tumour are required.

Clinical diagnosis of VHL disease can be confirmed by molecular analysis of the VHL gene and is informative in virtually all classic VHL families (families with multiple tumours) and classic sporadic VHL-patients (individuals with multiple VHL-related tumours).

Guidelines for clinical monitoring of VHL patients are presented. This monitoring protocol is recommended for carriers of a VHL germline mutation; members of VHL families with an unknown familial mutation; members of VHL families who decline testing for the familial mutation; and patients suspected of having VHL disease but without a VHL germline mutation.

Chapter 3, genetic investigations

Chapter 3 focuses on the clinical genetic and molecular genetic aspects of VHL disease. Section 3.1 describes VHL germline mutations and section 3.2 focuses on five families with a germline deletion of the VHL gene. Sections 3.3 and 3.4 report on case findings of VHL germline mutations in sporadic patients with a single type of VHL-related tumours.

VHL-germline mutations in the Netherlands

In the DNA laboratories of Utrecht and Rotterdam, VHL germline mutations were detected in 25 familial and seven sporadic VHL patients. We also identified VHL-germline mutations in two sporadic patients with VHL-related tumours who did not fulfil the current diagnostic criteria for VHL disease. Analyses of genotype-phenotype correlations were consistent with previous reports. Our study shows that *de novo* mutations represent at least 12% - and potentially 21% - of the germline mutations detected in the VHL gene in the present series, and provides evidence for non-penetrance and reduced penetrance of VHL germline mutations.

Five families with VHL germline deletions

We describe four families with partial deletions removing one or more exons of the VHL gene and one family with a deletion of the entire VHL gene. The deletions were detected by Southern blot analysis. In the fifth family, FISH analysis confirmed the deletion of the entire VHL gene. Our results showed that (quantitative) Southern blot analysis is a sensitive method for detecting germline deletions of the VHL gene and should be implemented in routine DNA diagnostics for VHL disease. Germline deletions in the studied patients were associated with a low risk for pheochromocytoma and a preponderance of CNS haemangioblastoma.

Patients with CNS haemangioblastoma-only

We report on the frequency of VHL germline mutations in 88 patients from the United Kingdom (n=63) and the Netherlands (n=25), with only CNS haemangioblastoma. A VHL germline mutation was found in three (3.6%) of 84 sporadic patients with a single haemangioblastoma and in two (50%) of the four sporadic patients with multiple haemangioblastoma. We concluded that VHL gene mutation analysis should be offered to all haemangioblastoma patients younger than 50 years. Further data are required to evaluate the detection rate in late-onset cases. The fact that we did not find a VHL gene germline mutation in two of the four patients with multiple haemangioblastoma may indicate, next to coincidence, the presence of additional haemangioblastoma susceptibility genes or alternatively, somatic mosaicism.

Patients and families with pheochromocytoma-only

We investigated the frequency of VHL germline mutations in Dutch patients with a pheochromocytoma-only phenotype. A total of 24 probands (14 with solitary, seven with multiple, bilateral or recurrent, and three with familial pheochromocytoma), were tested by molecular genetic analysis of the VHL gene. VHL germline mutations were not found in any of these probands with pheochromocytoma, even when features suggesting a germline mutation - such as early onset, multiple, recurrent, bilateral or familial tumours - were present. However, the absence of VHL germline mutations in the pheochromocytoma families may indicate the presence of additional pheochromocytoma susceptibility genes. Since mutation analysis of the VHL gene detects germline mutations in virtually all well-defined VHL families, we conclude that annual clinical monitoring for further VHL-related tumours in patients with pheochromocytoma and without a VHL germline mutation should not be recommended.

Chapter 4, discussion

In this chapter the principal findings of this research are discussed. This chapter focuses on the implications of the study and also provides conclusions and recommendations on some clinical and genetic aspects of VHL disease.

Carriers of a VHL germline mutation are predisposed for developing multiple tumours that often manifest at a relatively early age. In order to prevent both patient and doctor delay in the diagnosis of VHL disease, persons at risk for the disease as well as doctors should be provided with clear oral and written information about the clinical and genetic aspects of the disease. In addition, an intercentre co-operation should be established between the medical specialists involved to prevent unnecessary morbidity and mortality in patients with VHL disease. Multidisciplinary teams following national and international guidelines should guarantee the best results in the management of patients with VHL disease. A national VHL working group, led by a multidisciplinary board representing various institutes, has been established in the Netherlands to ensure uniform clinical management of VHL patients and families, as well as to carry out structural research projects. Clinical monitoring should be primarily organised around those VHL patients who have tested positive for a VHL germline mutation. To diminish the burden of frequent clinical surveillance, monitoring should

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be organised in a production line fashion so that all the necessary tests can be carried out during one hospital visit.

Although VHL germline mutations are identified in 100% of the classic families and patients with VHL disease, we report here on patients and families who exhibit some VHL characteristics but who do not carry a germline mutation in the VHL gene. Patients with multicentric, bilateral or familial VHL-related tumours and without a VHL germline mutation could play a role in identifying genes that are involved in their specific tumourigenesis. We demonstrate genotype-phenotype correlations for some tumours in VHL disease, but there appears to be no simple relationship between a germline mutation in the VHL gene and the manifestation of VHL-related tumours. For example, there is intrafamilial variability in the age of onset and the manifestation of different types of VHL-related tumours. There is evidence that genetic factors (so called modifier genes) and environmental influences play an additional role in the clinical expression of VHL germline mutations. Furthermore, we provide evidence of reduced penetrance and non-penetrance of certain VHL germline mutations.

It is not possible to accurately define the prevalence of VHL disease in the Netherlands from this study because of particular selection biases and incompleteness of information. However, our rough estimate of 1:64,000 will almost certainly prove to be higher and, interestingly, *de novo* mutations occur in a considerable proportion of all the families we studied. We present reasons why this group of patients is under-represented in our series and we demonstrate that *de novo* mutations represent at least 12% - and potentially 21% - of the VHL germline mutations detected in the Netherlands. In addition, we illustrated that VHL germline mutations can be identified in sporadic patients with VHL-related tumours who do not meet the current diagnostic criteria. These findings emphasise the importance of screening sporadic patients with one or more typical VHL-related tumours for germline mutations in the VHL gene. We suggest treating each patient suspected of having VHL disease, according to six categories we define here, with an open mind and performing: (1) an extensive pedigree analysis, (2) clinical screening for further VHL-related tumours, and (3) DNA analysis. Moreover, clinical situations leading to a suspicion of VHL disease should be eligible for DNA analysis in order to confirm, or exclude, the diagnosis of the disease since: (1) carriers of a VHL germline mutation and their relatives have a risk of developing multiple tumours; (2) molecular genetic analysis is readily feasible and identifies virtually all the classic VHL families and patients.

A principal finding of this study is that the early detection of VHL families and patients using molecular genetic analysis is effective, assuming that annual monitoring and timely treatment leads to a better prognosis for VHL patients. However, regarding the early detection of VHL patients, we observe that: (1) there is insufficient evidence of an improved quality of life or a longer life span; (2) there is no reliable analysis of the cost-effectiveness; and (3) the psychological consequences have not been studied sufficiently. These three observations should provide a basis for further clinical investigations. More extensive genetic research is indicated for clinical situations suggesting the presence of VHL disease, but without a VHL germline mutation.