



Mayo Clinic
Department of Medical Genetics
and the
VHL Family Alliance

Present the
**Fourth International Symposium
on von Hippel-Lindau**

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Abstract book for

VHL Care in the New Millennium

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Welcome

Dear Friends and Colleagues:

It is with great pleasure that we welcome you to the Fourth International Symposium on von Hippel-Lindau, to be held in Phillips Hall at the Mayo Clinic, 20-23 July 2000.

The Mayo Clinic is known throughout the world as a center of excellence in medicine. This conference brings experts from all over the world to share ideas and data, and to reach consensus on standards of care for people with von Hippel-Lindau.

Since the discovery of the gene for VHL in 1993, three Symposia have helped to accelerate progress in research and in the clinical understanding of VHL.

- Freiburg, 1994. Chaired by Dr. Hartmut Neumann
- Honolulu, 1996, chaired by Dr. Y. Edward Hsia
- Paris, 1998, chaired by Dr. Stéphane Richard

We look forward to your participation in the Mayo meeting, where we will look at ways of moving VHL care into a new era of less invasive management and more effective preventive medicine.

Conference co-chairpersons:

- Virginia Michels, M.D., Dept of Medical Genetics, Mayo Clinic
- Kelly Heselson, VHL Family Alliance

Dear Friends:

Your Minnesota VHL members, friends and families would like to extend a warm Minnesota Welcome to everyone. We thank you for taking time out of your busy schedule to attend the first conference in the year 2000.

We hope your visit is enjoyable and let us know if we can be of help in anyway. This conference will be successful if everyone will take time to introduce yourself to as many people as possible each day.

A big thank you to all the speakers who have volunteered their time to help us understand the VHL advancement steps that are taking place today.

-- Lois Erickson, Chairman, Minnesota VHL Chapter

Dear Friends:

The VHL Family Alliance is pleased to welcome you to this latest in our series of unique conferences focused on improving diagnosis, treatment, and quality of life for people affected by von Hippel-Lindau.

This a forum where medical professionals can share ideas and network together, forming working relationships that will persist long after this meeting.

This is also a setting where people with VHL may meet for the first time another person affected by this rare disorder. There will be a great deal of medical information, but there will be two things that have been hard to find for people with VHL. We hope that you will take away with you these two things:

- You are not alone.
- There is hope

There are other families and a community of very special professionals focused on making things better for you. Please collect your share of hugs and fun along with the information!

-- Peggy Marshall, Chairman, VHL Family Alliance

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U.S. Tax Deduction: The U.S. Internal Revenue Service has recently ruled that taxpayers may deduct some of the costs of attending medical meetings related to a dependent's health condition. For details, see IRS Revenue Ruling 2000-24 (pages 963-964) which can be found within IRS Revenue Bulletin 2000-19 (May 8, 2000). This issue is available online at http://www.irs.gov/bus_info/bullet.html

Abstracts	5
Basic Science	5
Structure of the VHL Gene and Protein	5
Function of the VHL Protein.....	5
VHL-regulated Gene Expression	7
Understanding and Controlling Tumorigenesis	9
Toward Gene Therapy.....	10
Genotype/Phenotype Alignment	12
Genetic Changes: Cause and Effect	12
Understanding Genotype/Phenotype Alignments	13
Finding All VHL Mutations.....	15
Introduction to Terminology and Concepts.....	16
VHL Care in the New Millennium.....	17
VHL Clinical Care Centers	17
Ethical, Legal, and Social Issues.....	18
DNA Testing.....	19
Optimal Clinical Care	20
Preserving Organ Function: Adrenals	23
Intraoperative Imaging	24
Achieving a Diagnosis	24
Preserving Organ Function: Kidney and Pancreas.....	25
Hemangioblastomas of the CNS and Retina	26
Stereotactic Radiosurgery for Hemangioblastoma.....	28
Sunday Meditation	29
Tumor Localization and Assessment	29
Radioactive Plaque Therapy for Large Eye Tumors	29
Innovative Eye Treatments and Clinical Outcomes	30
Progress in Research	33
Symposium Consensus Meeting	33
Forthcoming Meetings	34
Speaker Biographies	35
Index.....	45
Appendix A: Clinical Care Centers.....	47
Appendix B: Dealing with Medical Insurance	49

Abstracts

Basic Science

Structure of the VHL Gene and Protein

Molecular Image of the VHL Gene Product

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Mutation of the von Hippel-Lindau (VHL) tumor suppressor is associated with the inherited VHL cancer syndrome and the majority of kidney cancers in the general population. VHL binds the ElonginC and ElonginB proteins, forming the VCB ternary complex through which it regulates the cellular levels of angiogenic factors. The three-dimensional structure of this ternary complex provides a wealth of chemical information detailing the interactions between these three molecules, and reveals the spatial distribution of amino acids frequently mutated in cancer. Tumorigenic mutations are found to cluster in two surface patches on the molecule: a 35-residue domain of VHL responsible for ElonginC binding, and a separate domain of VHL remote from Elongin binding which is suggestive of a second macromolecular binding site. The structure extends the similarities to the SCF (Skp1-Cul1-F-box protein) ubiquitin ligase complex that targets proteins for degradation, supporting the hypothesis that VHL functions as a molecular adaptor in an analogous proteolytic pathway.

Nuclear/cytoplasmic localization of the VHL tumor suppressor gene and its importance for tumor suppression activity.

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Inactivating mutations of the von Hippel-Lindau (VHL) tumor suppressor gene cause the VHL cancer syndrome and sporadic renal clear cell carcinoma. VHL localizes mostly to the cytoplasm at steady state but engages in a dynamic transcription-dependent nucleocytoplasmic shuttle. VHL is unable to function as a negative regulator of Glut-1 when it is fused to a classical nuclear export sequence and induce to shuttle in a leptomycin B-sensitive, but transcription-insensitive, manner. Nuclear export of VHL-GFP in living cells requires ongoing RNA Polymerase II activity, and is mediated by mechanisms that are temperature-sensitive and energy-dependent. *In vitro* nuclear export of VHL-GFP is inhibited by nuclear pore-specific lectins, requires Ran and ATP-hydrolysis and occurs with kinetics that are similar to those of proteins containing a classical nuclear export signal. Size exclusion column chromatography and deletion mutant analyses suggest that VHL-GFP does not require assembly with one of its associated proteins, Cullin-2, to engage in nuclear export. The role of transcription-dependent shuttling plays in VHL ability to mediate oxygen-dependent degradation of Hypoxia-Inducible Factor- α will be discussed. These results suggest that sequences outside the Elongin C binding box may function as a nuclear export domain, potentially providing a novel role for this region of VHL frequently mutated in VHL disease.

Function of the VHL Protein

The VHL gene is involved in cross-talk between cell-ECM and cell-cell signaling

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Mutations of the von Hippel-Lindau (VHL) gene are involved in both the inherited family cancer syndrome and the development of sporadic renal cell cancer (RCC). Although reintroduction of VHL in VHL(-/-) cells can suppress RCC tumor cell growth *in vivo*, no effect on cells in standard culture conditions has been noted. To

examine the hypothesis that the tumor suppressor function of VHL requires signaling through contact with extracellular matrix (ECM), 786-0 RCC cells (VHL^{-/-}) and isogenic sublines stably expressing the VHL gene products were grown on Matrigel and collagen I. Differences in morphology were noted between VHL (+) and (-) cell lines within 12 hours of plating that were dependent on cell density prior to plating the cells on these matrices. The VHL(+) lines differentiated into organized epithelial sheets whereas the VHL(-) lines were branched and disorganized. Strikingly, VHL (+) cells grown to high density on collagen I became growth arrested whereas VHL (-) cells continued to proliferate. FACS analysis indicates that the VHL(+) lines become arrested in G₀/G₁, while the VHL(-) lines had S phases of up to 40%. In addition, pRB was hyperphosphorylated in the VHL (-), but not the VHL(+) lines. Biochemical markers of differentiated proximal tubule cells were upregulated in VHL(+) cells, and growth at high cell density enhanced the effect. Thus, VHL protein mediates growth arrest and differentiation through integration of cell-cell and cell-ECM signals.

The von Hippel-Lindau tumour suppressor is a component of an SCF-like E3 Ubiquitin-protein ligase complex

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The ubiquitination and subsequent degradation of proteins is a strictly regulated process. Loss of regulation, leading to the accumulation of such proteins as pro-angiogenic factors, can predispose a cell to tumorigenesis. We find that pVHL, the product of the VHL tumour suppressor gene, exists *in vivo* in an E3 ligase complex that displays ubiquitination promoting activity in conjunction with the universally required components E1, E2 and ubiquitin. pVHL-associated ubiquitination activity is dependent on binding of pVHL to elongin C and CUL-2. More importantly, tumour-derived mutants of pVHL demonstrate loss of associated ubiquitination promoting activity, suggesting a direct link between pVHL's role in tumour suppression and the process of protein ubiquitination. Accordingly, these results identify a biochemical function for pVHL as a component of an E3 ubiquitin protein ligase complex, targeting specific cellular proteins for destruction. The identification of proteins normally selected by pVHL for degradation will begin to broaden our understanding of the variety of pathways controlled by this tumour suppressor.

Molecular Pathogenesis of the von Hippel-Lindau Hereditary Cancer Syndrome

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The protein product of the von Hippel-Lindau gene, pVHL, does not closely resemble other known proteins. It does, however, form multimeric complexes containing elongin B, elongin C, Cul2, and Rbx1. These complexes resemble so-called SCF complexes (Skp1/Cdc53/F-box protein) that function as E3 ubiquitin ligases. Thus, by analogy, pVHL was suspected to play a role in protein ubiquitination. Recent work from Peter Ratcliffe's group (Oxford, UK) showed that cells lacking pVHL can not degrade members of the HIF (hypoxia-inducible factor) transcription factor family. We have gone on to show that HIF is, indeed, ubiquitinated in a pVHL-dependent manner. A frequently mutated region of pVHL binds directly to HIF and is required for HIF ubiquitination *in vitro* and in cells. pVHL recognizes a region of HIF previously implicated in oxygen-dependent protein turnover. Thus, in normal cells HIF is only stable under hypoxic conditions. In cells lacking pVHL, it is stable irrespective of ambient oxygen levels. This accounts for the earlier observation that cells lacking pVHL accumulate high levels of hypoxia-inducible mRNAs under well-oxygenated conditions.

Among these mRNAs are mRNAs encoding proteins such as VEGF and PDGF B that almost certainly contribute to the vascular nature of VHL-associated neoplasms. We are currently exploring the possibility of developing drugs that will either inhibit HIF or will inhibit certain downstream HIF target genes such as VEGF.

VHL-regulated Gene Expression

Serial Analysis of Gene Expression to identify targets for therapeutic use

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Von Hippel Lindau (VHL) syndrome has been recognized as a distinct disease for about 100 years. Progress in understanding VHL has come slowly for most of this time and has mainly involved description of the many manifestations of the disease in patients. In 1993, a tremendous amount of work led to the identification of a single gene that is responsible for VHL disease when it is mutated. Since that time, steady progress in VHL research has provided a glimpse of how the VHL protein functions in a cell. An important discovery is that VHL is likely important for directing the cell to eliminate other proteins. One very important example of this is the protein HIF-1. HIF-1 is a transcription factor whose purpose is to activate other genes. At least one of the genes activated by HIF-1 is VEGF, a critical gene for recruiting blood vessels to tissue in need of oxygen. Therefore, if VHL is inactive, HIF-1 would not be destroyed appropriately and VEGF would be continuously present. This is a clear example of how VHL serves to regulate the expression of genes in the cell. We believe that VHL may affect the regulation of many additional genes by targeting regulatory proteins, like HIF-1, for destruction. In the absence of proper VHL function many genes may lose their proper controls and are expressed inappropriately. Learning which genes lose their proper regulation when VHL function is absent would be an important step in identifying new targets for treatment of VHL disease. We have used a technique called SAGE (short for Serial Analysis of Gene Expression) that allows us to examine how the overall pattern of gene expression changes in a cell when VHL is present or absent. Currently, we have examined thousands of expressed genes in renal carcinoma cells from VHL patients. Our lab is currently screening these expression patterns for differences that occur as a result of VHL being present or absent. Using these expression profiles, we will continue to focus our efforts on identification of promising targets for therapeutic use against VHL disease.

The role of the VHL gene in neuronal differentiation in human neuroblastoma

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The von Hippel-Lindau (VHL) gene has an important control mechanism for tumorigenesis, angiogenesis and embryogenesis. VHL gene and protein are expressed in neuronal cells of fetus and adult brain. However, the role of VHL gene in the central nervous system (CNS) has not been elucidated. We investigated the role of the VHL gene in neuronal differentiation using human neuroblastoma cells. The exogenous expression of VHL protein induced neuronal differentiation of neuroblastoma morphologically, biochemically and electrophysiologically, while the inhibition of endogenous expression of the VHL protein showed the decline of neuronal character.

In conclusion, VHL gene plays an essential role in neuronal differentiation in neuroblastoma.

The role of the VHL protein in control of oxygen-regulated VEGF gene expression.

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VHL-related tumors, including renal cell carcinoma (RCC), over-express vascular endothelial growth and permeability factor (VEGF) as a result of inactivation of the VHL protein (pVHL). VEGF over-expression may solely account for the highly vascular presentation of VHL-related tumors. Understanding the mechanism(s) of regulation of VEGF expression has been a major focus of VHL research, since targeting of VEGF expression and activity may hold great potential in future biological or genetic therapies. VEGF gene expression is

controlled transcriptionally via SP1 and Hypoxia Inducible transcription factors. In addition, VEGF mRNAs are rapidly degraded through multiple stability elements, including AU-rich elements (ARE) in the 3' untranslated region. However, under conditions of hypoxia, VEGF mRNAs are stabilized. VEGF mRNA decay and stability are mediated through ARE-binding protein complexes, a process that we have found to be controlled by pVHL in RCC cell lines.

The VHL tumour suppressor: role in oxygen regulated gene expression.

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Matching oxygen consumption and supply represents an important challenge to multicellular organisms, and the relationship is disturbed in many pathological processes. HIF-1 is a transcription complex which is emerging as a key mediator of oxygen homeostasis. This complex, which contains an α and β subunit (both basic helix-loop-helix proteins of the PAS family) is formed in hypoxia and modulates expression of a broad range of genes involved in cellular processes such as angiogenesis and cellular metabolism. Regulation involves ubiquitin-mediated oxygen-dependent destruction of the alpha subunit.

Our group has shown that pVHL is a component of HIF-1 complexes and is necessary for oxygen-dependent proteolysis of the alpha subunit, with pVHL involved as the recognition component of the E3 ubiquitin ligase complex. In vivo metabolic display of pVHL associated proteasomal substrates reveals only HIF α subunits, suggesting a high degree of specificity for these targets. Interaction involves the pVHL β domain and the oxygen dependent degradation domain of HIF α . The ability of pVHL to ubiquitylate HIF is prevented by mutations in the β domain (which prevent target capture) and by mutations affecting Elongin BC binding, supporting the importance of HIF activation in the VHL syndrome.

Regulation of tyrosine hydroxylase gene expression by VHL tumor suppressor complex in pheochromocytoma PC12 cells.

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Tyrosine Hydroxylase (TH) is the rate-limiting enzyme in catecholamine synthesis, expressed in a tissue specific manner in catecholaminergic neurons in the central and peripheral nervous system, and in chromaffin cells of adrenal medulla and carotid body. TH belongs to the group hypoxia-regulated genes, and is regulated by hypoxia at the level of transcription and mRNA stability. Hypoxia stimulates TH transcription through HIF and AP-1 binding transcription factors. Tumors derived from the adrenal medulla chromaffin cells, pheochromocytomas, express high levels of TH protein and mRNA. This results in increased biosynthesis and release of catecholamines, and arterial hypertension, the main clinical symptoms of pheochromocytoma tumors. Familial form of pheochromocytoma tumors occurs in the VHL disease. Our laboratory has demonstrated that pVhl tumor suppressor protein is a potent *negative regulator* of TH gene transcription. Overexpression of pVhl in rat pheochromocytoma derived PC12 cells represses expression of TH gene by inhibiting elongation of TH transcript in the downstream region of the TH gene. Increased levels of pVhl result also in substantial attenuation of hypoxic accumulation of TH mRNA. This is due to the inhibition of the hypoxic induction of the TH promoter activity by pVhl. Decrease in the pVhl levels due to the expression of pVhl antisense RNA results in increased expression of TH mRNA and TH transcription as measured by the nuclear run-on assays. The work of our laboratory is concentrated on elucidating the molecular mechanisms involved in regulation of TH transcription by pVhl during normoxia and hypoxia.

Understanding and Controlling Tumorigenesis

The VHL knockout mouse: A tool for understanding oxygen-regulated development.

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In our attempts to establish an animal model for VHL disease, we have generated a mouse line with one copy of the VHL gene deleted. Embryos that contain no functional VHL (-/-) die because of placental failure at mid-gestation, at a stage of development in which cytotrophoblasts (specialized placental epithelial cells) normally differentiate and invade the uterus and its vasculature. Previous studies in renal cancer cell lines showed that pVHL inactivation led to loss of normal controls of hypoxia-responsive gene products and a more motile and invasive phenotype. Since normal placental development is regulated by oxygen tension and cytotrophoblast motility and invasion resembles in some ways tumor cell invasion, we evaluated the role for pVHL in these processes in normal and VHL -/- placentas and cytotrophoblasts. We have found that cytotrophoblast differentiation and invasion is similarly controlled through regulation of pVHL expression.

Progress toward the development of a mouse model for VHL disease

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The development of a mouse model for VHL disease will allow analysis of the effects of VHL protein deficiency on cell growth and differentiation. We propose to circumvent the embryonic lethality produced in VHL knockout mice by developing a conditional VHL knockout mouse using Cre/lox site specific recombination technology. Cre recombinase recognizes and deletes DNA sequences between two specific 34 bp lox-P sites. A vector in which the target gene is flanked by lox-P sites (*floxed*) is introduced into embryonic stem (ES) cells by homologous recombination. The *floxed* mice from these ES cells are crossed with mice carrying the Cre recombinase under a tissue-specific or time-dependent promoter, resulting in deletion of the targeted gene in a time- or tissue- specific manner. We have constructed a target vector in which exons 2 and 3 of the mouse VHL gene are *floxed*. After homologous recombination, three ES cell clones contained the VHL*flox*/+ genotype by Southern analysis and were injected into lastocysts to generate chimeric mice and test for germline transmission. The VHL*flox*/+ ES cells will also be used to produce VHL*flox/flox* and VHL*null*/+ ES clones by transient Cre expression *in vitro*. We plan to cross VHL*flox/flox* mice derived from these clones with VHL*null*/+ mice carrying the Cre recombinase under a kidney -specific promoter. Cre expression will result in deletion of the VHL gene only in the kidney cells. The conditional VHL knockout mouse will provide a model of human VHL disease to study kidney tumor initiation and progression and to test gene therapies.

Evidence for a Stepwise Progression Model for VHL Tumorigenesis

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Cytogenetic abnormalities and high frequency loss of heterozygosity (LOH) have been documented within the short arm of human chromosome 3 in the nonpapillary form of sporadic renal cell carcinoma. The *VHL* gene at 3p25 has been identified as a tumor suppressor gene for RCC. Functional studies as well as molecular genetic and cytogenetic analyses have suggested as many as two or three additional regions of 3p could contain tumor suppressor genes for sporadic RCC. We have previously functionally defined a novel genetic locus *NRC-1* within chromosome 3p12, distinct from the *VHL* gene, that mediates tumor suppression and rapid cell death of renal cell carcinoma cells *in vivo*.

We report the tumor suppression of RCC cells *in vivo* following the transfer of a defined centric 3p fragment (encompassing chromosome 3p12-q11) into different histologic types of RCC. Results document the functional involvement of *NRC-1* in not only different cell types of RCC (i.e. clear cell, mixed granular cell/clear cell, and

sarcomatoid types), but also in papillary RCC, a less frequent histologic type of RCC for which chromosome 3p LOH and genetic aberrations have only rarely been observed.

We also report that the tumor suppression observed in functional genetic screens was independent of the microenvironment of the tumor, further supporting a role for *NRC-1* as a more general mediator of *in vivo* growth control. Furthermore, *NRC-1* mediates tumor suppression irrespective of the presence of a mutation in the von Hippel Lindau (*VHL*) tumor suppressor gene.

Thus, these data provide the first functional evidence for a *VHL*-independent pathway to tumorigenesis in the kidney via the genetic locus *NRC-1*. In addition, high frequency loss of heterozygosity (LOH) in *VHL*-associated and sporadic pancreatic islet cell tumors was documented within chromosome 3p. These data indicate that LOH for 3p loci proximal to *VHL* correlates with malignant conversion in *VHL* disease and implicate a stepwise model for malignant conversion in *VHL* disease.

VHL Gene targeted by Carcinogens

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In the past, trichloroethylene (TRI) was used for metal cleaning in various industries. After latency periods of up to 30 years, possible health hazard concerns to formerly exposed individuals have emerged. In particular, an increased renal cancer incidence was observed among individuals with high dose occupational TRI exposures. Since sporadic clear cell renal cell carcinoma is associated with somatic *VHL* gene mutations we set out to document a possible relationship between TRI exposure, *VHL* gene damage and the occurrence of kidney cancer. We recruited 44 kidney cancer patients formerly exposed to TRI on their jobs. Exposure levels were assessed by questionnaire and scored into three intensity levels (high, medium, and low) based on the clinical symptoms, the duration and frequency of working with TRI, and the prevalent way of handling liquid TRI. Also, *VHL* mutation characteristics were established.

Thirty-three patients (75%) with somatic *VHL* mutations had high and medium exposures. The tumors showed a unique *VHL* mutation pattern. In particular, a frequent mutation (C>T at nucleotide 454) was observed in 13 patients (39%) and in normal tissue of four cases. Cytosines were preferentially involved at mutation sites. Multiple *VHL* mutations, were observed in 14 patients (42%). Altogether there was a statistically significant association ($p=0.0006$) between the severity of TRI exposure and the multiplicity of mutations.

Our findings represent first molecular evidence for a relationship between exposure to a defined carcinogen, gene damage, and kidney cancer

Toward Gene Therapy

Cancer Gene Therapy with an Adenoviral Vector Expressing VHL Tumor Suppressor Gene

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Loss of Von Hippel-Lindau (*VHL*) tumor suppressor gene function can give rise to familial forms of many cancers as well as sporadic forms of some cancers including renal cell carcinoma and pancreatic cancer. The goal of this project is to investigate a cancer gene therapy approach using an adenovirus expressing *VHL* gene (*AdVHL*). Our initial studies using renal (786-0, A-498, Caki, ACHN) and pancreatic cancer cells (PCI-6, PCI-10, PCI-19, PCI-24, PCI-35, PCI-43) showed that the cells which have lost *VHL* functions, express high levels of Hypoxia-inducible factors, known to regulate the expression of pro-angiogenic molecules such as vascular endothelial derived growth factors. To investigate the feasibility of using angiogenesis as the target for gene therapy, we showed that infection of human renal cancer cells, with a recombinant adenoviral vector expressing

β -galactosidase, resulted in high level transgene expression. To investigate *in vivo* efficacy of AdvHL, 786-0 cells were injected subcutaneously in nude mice (10^7 cells/animal), and palpable tumors appeared in 10 days. In this xenograft model 786-0 cells induced massive angiogenesis. We next injected AdvHL directly into the pre-established kidney tumors (2×10^8 pfu in 0.1-ml). Interestingly, two out of six animals, which received AdvHL, showed initial complete tumor regressions, while none of the control animals, which received buffer alone or AdNull showed any regression. These results suggest the use of AdvHL as a viable gene therapy strategy for renal cell carcinoma and deserve further investigation.

Transmembrane carbonic anhydrases as new targets for cancer treatment

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We identified and characterized two new targets of pVHL: transmembrane carbonic anhydrases CA9 and CA12. The products of these genes are enzymes involved in reversible reaction of carbon dioxide hydration. Both isoenzymes are highly overexpressed in most cancers studied and may serve as molecular markers for some of them: kidney, CNS, lung and colonic tumors. Both genes may be up-regulated by hypoxia and their overexpression in VHL-deficient cells *in vitro* correlate with acidification of extracellular media. Thus, we suggest that activation of these genes is an inherent feature of cancer cell metabolism.

Bicarbonate production catalyzed by CA9/CA12 may be crucial for the cells experiencing glycolytic acidification (Warburg effect). Both enzymes may also contribute to the invasive capacity of cancer cells by activating metal-dependent proteinases via acidification. Thus, treatment of kidney, brain and lung tumors with carbonic anhydrase inhibitors (acetazolamide, benzolamide, etc.) may not only impair cancer cells ability to withstand hypoxia and metabolic acidification but also suppress metastasis. The proteins may also be used as markers for hypoxic cancer cells. Funded by NCI, NIH, Contract No. NO1-CO-56000.

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Monoclonal antibodies for the treatment of kidney cancer: relationship between VHL and antigen expression.

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Monoclonal antibodies (mAbs) preferentially reactive with tumor cells could bypass the main disadvantages of chemotherapeutics and biological response modifiers: drug-resistance and non-target organ toxicity. In addition, they may provide new therapeutics for malignancies for which no therapy is available. In search for monoclonal antibodies (mAbs) discriminating between normal kidney tissue and renal cancer, we isolated mAbG250, reactive with a membrane-associated antigen of renal cell carcinoma (RCC). In a number of clinical trials we tested the radioimmunotherapeutic approach with ^{131}I -labeled chimeric G250, injected intravenously (i.v.). Additionally, a trial with unmodified mAbG250 was initiated.

MABG250 reacts with all clear cell carcinomas of the kidney, highly suggestive for a causal relationship with VHL. However, studies to link G250 expression with VHL were not possible because the G250 antigen was not molecularly identified. Recently, we have molecularly defined the G250 antigen and found that it is identical with CAIX, initially defined as a cervical cancer-associated antigen. Introduction of wildtype VHL leads to complete abolishment of G250 expression and promoter studies show that introduction of VHL^{wt} downregulates G250 promoter expression. Thus, a clear link between VHL and G250 expression exists. We have shown that one of the consensus elements of the transcriptional activator Sp1 in the G250 promoter is quintessential for the

G250 promoter function. It is feasible that VHL downregulates G250 transcription by directly binding and inhibiting Sp1, similar to vascular endothelial growth factor transcript

Genotype/Phenotype Alignment

Genetic Changes: Cause and Effect

Phenotypic Variability in VHL disease: Allelic Heterogeneity and Genetic Modifiers

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A characteristic feature of VHL disease is marked interfamilial and intrafamilial variability in phenotypic expression. Interfamilial differences in specific tumour risk (e.g. pheochromocytoma susceptibility) reflect allelic heterogeneity. In addition we have reported evidence of genetic modifiers in VHL disease (Webster et al. *American Journal of Human Genetics* 1998;63:1025-1035) and have continued to investigate the molecular basis of phenotypic variability in VHL disease.

First Generation Diagnoses of von Hippel-Lindau Syndrome: New Mutations and VHL Mosaicism.

Glenn G, Stolle C*, Sgambati M, Middleton L, Choyke P, and Linehan WM.

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Individuals affected by VHL were analyzed by clinical and genetic methods. We screened for clear cell renal carcinomas, pheochromocytomas, pancreatic cysts and neuroendocrine tumors, brain and spine hemangioblastomas, retinal angiomas, endolymphatic sac tumors, and epididymal cystadenomas. Molecular analyses of the *VHL* gene were by standard methods of Southern blot analysis and DNA sequencing, (Stolle et al, 1998). In special cases supplemental methods included CSGE (conformational sensitive gel electrophoresis) and DNA sequencing of cloned genomic DNA (Sgambati et al, 2000) and FISH (fluorescence in situ hybridization) (Pack et al, 1999).

VHL mutations were identified in individuals from 166 kindred, of which 42 (25%) had no prior VHL family history. To determine whether these 42 represent new germline mutations, we began testing parents. For 11 of 42, both parents were negative for the mutation in their affected offspring. For 14 both parents are living but untested. For 15 one or both parents are deceased. For 2 of 42 (ca. 5%), each had a parent with detectable VHL tumors and cysts, but no *VHL* mutation detectable by routine testing. When additional methodologies were used to analyze blood and other tissues of these parents, a portion of their tissues carried identical *VHL* mutations as their offspring. Currently, five additional unrelated individuals, having VHL tumors but no detectable *VHL* mutation, are being studied for possible *VHL* mosaicism. Understanding mosaicism and its relationship to new germline mutations is important. Developing feasible testing strategies may identify individuals at risk who may benefit by screening for clinical disease.

First Generation Diagnoses in VHL: Abstract 3-17-00, GMG

Somatic Mosaicism in VHL and its Implication

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The occurrence of somatic mosaicism in VHL disease was recently reported by two independent groups (A.Murgia et al. *Human Mutation*, Mutation in Brief #279 1999; M.T. Sgambati et al *Am.J.Hum.Genet.* 2000.) In our case somatic mosaicism has been detected in an asymptomatic 48 year-old woman, mother of a severely

affected VHL patient. A complete clinical investigation, entirely based on the molecular data collected lead to the identification of renal cysts and pheochromocytoma in the mosaic individual; she was totally negative, at funduscopy and MRI, for the presence of hemangioblastomas that had been found in her affected son at a much younger age.

Somatic mosaicism is a well known phenomenon described for a number of disorders among which familial retinoblastoma, NF2 and NF1 for which has been hypothesized that somatic mosaicism may be a relatively frequent condition, and that such a condition might mediate the expressivity and penetrance of the disease. The same seems to be true for our case, characterized by a rather late age of onset and mild phenotype.

Somatic mosaicism is a possible occurrence in VHL disease. This phenomenon could at least partly provide an explanation for the observed variable phenotype severity, and definitely increase the difficulty of a correct diagnosis of this multisystem disease. Somatic mosaicism may be very difficult to suspect on merely clinical ground, but it should always be considered in parents of affected individuals. This will have a direct impact on clinical practice, improving genetic counseling, in terms of prediction of phenotype and risk of recurrence, and will contribute to generate better data on the actual incidence of VHL de novo mutations.

Is P25L a "real" VHL Mutation?

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The VHL gene has two translational initiation sites in exon 1, separated by 53 codons. Both proteins have been detected in cells and have equivalent activity. A mutation in the first 53 codons of the upstream open reading frame will have no effect on the structure of the smaller downstream protein. As expected, the vast majority of VHL mutations that have been found are downstream of the second initiation site and alter both proteins. However, several candidate mutations have been found in the first 53 codons.

We have found an individual with apparent VHL disease (multiple CNS and retinal hemangioblastomas) with two VHL gene mutations, P25L and P86R. P25L, which has been reported previously in a person with pheochromocytoma, alters the upstream protein only. P86R has also been reported previously and alters both VHL proteins. Her 16 year old son, who has not yet developed symptoms of VHL, has the P86R mutation, but not P25L. We suspected, based on the positions of the mutations, that P86R is pathogenically significant, but have doubts about the significance of the P25L mutation. For this reason we carried out a survey of anonymized DNAs for P25L, which showed that it is a variant with an allele frequency of about 0.5%. However, our work does not prove that P25L is entirely innocuous.

Understanding Genotype/Phenotype Alignments

VHL Genotype Studies in France: Medical and Fundamental Implications

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As early treatment of VHL manifestations significantly improves the clinical outcome and the quality of life of patients, early and unambiguous diagnosis is mandatory. Genetic testing is emerging as a major tool now that virtually all VHL gene germline mutations are detectable. A French coordinating working group was formally organized in 1996, in order to improve early detection and clinical management of VHL families. Molecular diagnosis is currently performed in four Laboratories after a specific consultation with a clinical geneticist and obtaining of informed consent.

To date, a VHL germline mutation has been identified in 119 distinct VHL families including 401 patients, allowing presymptomatic diagnosis in at-risk family members. Subsequently, 44 asymptomatic gene-carriers

were detected, 14 of them (10-59 years) having tumors to be treated, and 115 individuals were demonstrated to be unaffected. The age at which genetic testing should be performed in VHL families remains debated. The minimal age would seem to be 5 years of age since no manifestation of the disease has been detected before that age in our series. In addition, we found a germline mutation in some patients with sporadic tumors including CNS hemangioblastoma (2/43), retinal hemangioblastoma (2/14), and renal cell carcinoma (1/17), leading to propose systematic familial testing.

On the other hand, the large series of patients with an identified VHL germline mutation (more than 30 gene-carriers for some mutations) provided a basis for more precise genotype-phenotype correlations.

Renal lesions in VHL disease : toward genotype-phenotype correlation

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Germline mutation in the VHL gene may be detected in 80-100% of the families with VHL disease. Although an important inter- and intrafamilial variability of renal involvement has been observed, no accurate assessment between mutations and clinical features has been reported. In an attempt to better delineate genotype-phenotype correlation, we reviewed renal lesions in 189 patients from 86 VHL families with VHL germline mutations characterized by SSCP or Southern-blotting. According to CT and clinical involvement, renal disease was reported as 1) mild : single cyst, or polycystic kidney, or undetermined or solid lesion without indication for surgery 2) moderate : less than 5 solid lesions affecting one or both kidneys, with > 1 tumor requiring surgical treatment 3) severe : solid lesions before 25 years of age, or > 5 solid lesions within one or both kidneys, or metastasis of renal carcinoma. Genotype-phenotype correlations are currently evaluated according to the variety of gene alteration, and the functional consequence on the VHL protein.

Roles of genetic polymorphisms in the incidence and prognosis in Japanese patients with sporadic RCC.

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Germ-line mutation in the von Hippel-Lindau (VHL) tumor suppressor gene predispose a hereditary VHL disease patient to develop renal cell carcinoma (RCC). This is also the case with non-hereditary sporadic RCC and somatic mutation of VHL gene has been also detected in approximately 80% of sporadic RCC tumors.

But, over all about 30% of VHL patients develop RCC, and tumors occasionally regress spontaneously in sporadic RCC patients. These findings suggest that besides mutation of the VHL gene, other factors such as genetic polymorphisms or immunological responses of hosts influence the development and progression of RCC. Recently, it was reported that two types of genetic variants existed on the IL-4R α gene were associated with the development of atopy by predisposing the host immune system to Th2. To examine the roles of Th1/Th2 immune response to RCC, we have analyzed two polymorphic sites of the IL-4R α gene: the substitution of valine (Val) to isoleucine (Ile) and glutamine (Gln) to arginine (Arg) among 143 cases of sporadic RCC in Japanese populations. It was revealed that the frequency of Th2 dominant alleles was significantly higher in the RCC population than in healthy controls with OR of 1.52 ((Ile/Ile+Ile/Val versus Val/Val) 95% CI 1.01-2.30; p=0.046) and 1.68 ((Arg/Arg+Arg/Gln versus Gln/Gln) 95% CI 1.09-2.61; p=0.019). Furthermore, the patients with Ile/Ile presented a significantly lower cause-specific survival (p =0.023). These results suggest the possibility that genetic polymorphisms of the IL-4R α gene also play significant roles in the development and progression of VHL-RCC.

Pheochromocytomas in VHL patients

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Pheochromocytomas are present in 11-19% of VHL patients. Based on the presence of pheochromocytomas, VHL is further subdivided into two types: type 1 (without pheochromocytomas) or type 2 (with pheochromocytomas). Missense mutations of the VHL gene are associated with a higher risk of pheochromocytomas than truncating mutations. Here we analysed clinical and genetic data in a series of 102 VHL patients with pheochromocytomas from 62 different families.

Pheochromocytoma was the first manifestation of VHL disease in 58 patients and the sole manifestation in 7 families; the average age at diagnosis was 27 years (5-74); 20 patients (20%) were less than 16 years old. Adrenal and extra-adrenal pheochromocytomas affected 92 and 12 patients respectively. Of the 92 adrenal pheochromocytomas, 48 were bilateral (52%).

We identified 34 different VHL gene mutations in 54 families and found missense mutations in 79 patients (77.4%) from 43 families. Fifty seven patients had missense mutations located in the region coding for the alpha domain of VHL protein.

Pheochromocytomas were present in 69% of missense mutations carriers but in 35% of truncating mutations carriers.

Genetic diagnosis of the VHL disease allows accurate presymptomatic diagnosis and pheochromocytomas risk assessment. Screening of pheochromocytomas should start precociously and life-long follow-up should be carried out in missense mutation carriers.

Finding All VHL Mutations

VHL Testing

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The characterization of new disease-genes and the diagnostic application of molecular technologies have an enormous impact on clinical practice through the extended provision of molecular diagnosis to a constantly growing number of disorders. Very often though, even in cases of well-known monogenic diseases molecular diagnostics is limited by a low mutation detection rate. This can be due to the complexity of the gene, to the frequency of new mutations, or to other factors such as the location of disease-causing mutations outside the coding sequence or a condition of somatic mosaicism.

VHL mutation scanning is performed in our laboratory with quantitative Southern blot, SSCP analysis and direct sequencing. This strategy has led to the identification of disease-causing mutations in 15 out of 16 cases of clinically confirmed von Hippel-Lindau disease. Germline VHL mutations have been detected in 2 cases of isolated pheochromocytoma and 1 case of isolated CNS haemangioblastoma, all with negative family history. One case of somatic mosaicism has been detected and a few intronic variants, found in patients with isolated VHL-related tumors, are currently being characterized

The molecular protocol we have used has allowed the detection of mutations in 94% of the VHL patients tested. The identification of the mosaic and the fact that the only apparently molecular negative VHL of our series is a sporadic case, suggest a modification of the current test to increase the sensitivity for the presence of a possible low-grade mosaicism. Analysis of the VHL mRNA should be performed in cases in which intronic variants, even if not altering invariant splice site nucleotides are found, to exclude a modification of the VHL transcript.

Genetic Diagnostic Testing for von Hippel-Lindau Disease

Catherine A. Stolle, Ph.D.

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VHL is an autosomal dominant disorder that predisposes to specific tumors of the brain, spine, eye, kidneys, adrenal glands, pancreas, inner ear, and broad ligament and epididymis. The VHL gene was localized to the short arm of chromosome 3 in 1988 (Seizinger et al.) and isolated by positional cloning in 1993 (Latif et al.). Since the discovery of the gene and detection of the first mutations that cause VHL, we have been working on assays to detect 100% of germline mutations¹ in patients with VHL. Recently, in conjunction with colleagues at the National Cancer Institute, we achieved that goal using a combination of quantitative Southern blot analysis, conformation sensitive gel electrophoresis (CSGE), and DNA sequence analysis (Stolle et al., 1998).

Quantitative Southern blot analysis is used to detect large mutations involving many thousands of DNA bases (i.e., partial or complete deletion of the DNA region that comprises the VHL gene). This technique detects mutations in ~28% of patients with VHL and is usually performed first on patients without a known mutation. The second phase of testing involves polymerase chain reaction (PCR) amplification of exons 1-3 of the VHL gene and a mutation scanning assay (CSGE) to detect exons that contain small mutations (i.e., 1 to several bases). DNA sequence analysis is then performed to identify or rule out a mutation.

This approach has been successfully used to find mutations in 142 out of 142 consecutive VHL patients evaluated at the NCI, and can be used to rule in or rule out a germline mutation in patients with symptoms suggestive of VHL. Discussion will focus on the types of mutations that cause VHL, the assays used to detect these mutations, and the lessons learned from some unusual cases.

1. Mutations present in every cell of the body, in contrast to sporadic mutations that may be present in only some cells.

References:

Seizinger et al. Nature 332: 268-269, 1988.

Latif et al. Science 260: 1317-1320, 1993.

Stolle et al. Human Mutation 12: 417-423, 1998.

Introduction to Terminology and Concepts

VHL 101: Introduction to Terminology and Concepts

Joyce Graff, Editor, *VHL Family Forum*

VHL Family Alliance, Brookline, Massachusetts

People who live with VHL over many years necessarily become familiar with many medical terms that come up in conversation with their physicians. Nonetheless, one individual with VHL will not be exposed to the terminology in all the many fields of specialization that touch on the syndrome, and people who have never before been to a VHL meeting, or people who are new to VHL, may find the vocabulary somewhat daunting.

For this reason, the VHL Family Alliance has offered this introduction to the vocabulary and concepts that will come up in the course of the meeting. The VHL Handbook contains a glossary of terms that will be useful to keep at hand,

This gathering on Friday morning is also an opportunity to meet some of the other first-timers, and some of the members of the Board who will be available for questions over the course of the meeting.

VHL Care in the New Millennium

VHL Care in the New Millennium: the Role of Genetics

Virginia V. Michels, MD

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The Human Genome Project was conceived in the 1980's as a plan to sequence all the genetic material found in humans.

This year, far ahead of the originally scheduled completion date of 2005, the first raw sequences have been accomplished. What does this really mean? "Raw" means that there remain gaps in the sequence and that some of the sequences are out of order. Although this is a remarkable accomplishment, it does not tell us all we need to know about genetic diseases. The next step is to annotate the genome, ie. to find the genes' start and stop sites, to determine the genes' structures, and to figure out what they do. The next big project will be Proteomics, the identification and characterization of all the proteins that are made by these genes. This is an enormous task, because even though each of our genes is present in all our cells, the expression of these genes into proteins varies tremendously from one organ to the next. To make things even more complicated, even in the same organ, genes get turned on and off at various times. The understanding of all this coordination between genes and proteins is only beginning to be understood for only a few genes.

Is any of this important for VHL, when we already know the gene responsible, the protein it makes and some of its functions? I will give several reasons why I believe it is very important, and how it may impact medical care of people with VHL in the coming millenium.

VHL Clinical Care Centers

VHL Clinical Care Centers: Who is Participating, Goals of the Program.

Joyce Graff, Editor and CCC Chair

VHL Family Alliance, Brookline, Massachusetts

The goals of the Clinical Care Program of the VHL Family Alliance are:

- To improve diagnosis and treatment of VHL
- To provide coordination of care across medical specialties
- To provide resource centers for patients and physicians who are new to VHL
- To provide a ready channel for communicating advances to these centers of expertise
- To provide a model which can be replicated elsewhere.

Standards of care have been developed by the Medical Advisory Board of the VHL Family Alliance in cooperation with the participating institutions. The initial recommendations are embodied in the VHL Patient Handbook, available from the VHL Family Alliance.

Cooperating institutions have agreed that they will designate a single point-of-entry into the institution where the term VHL will be recognized and staff will know how to assist. They will take responsibility for helping a patient file all the needed specialists, and check all the appropriate areas of the body which need screening. They will ensure communication among the specialists involved in a patient's care, and wherever possible will do their best to coordinate appointments to minimize the time the patient and family need to spend at the center.

These centers may also serve as a source of second-opinions, or referrals from Health Maintenance Organizations and physicians less familiar with VHL.

See Appendix A for a list of participating institutions.

For additional information or to apply to join the Clinical Care program, please contact 1-617-277-5667 or vhccc@vhl.org

Summarization of 4-year activity of Polish VHL Registry

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Aim: To summarize results of "active" search of VHL patients and their management.

Patients and methods: Our Registry comprises two groups of patients:

1. group I -- 19 patients from 13 families with VHL diagnosis established before 1996
2. group II -- 51 patients from 26 families with VHL diagnosis after 1996, that is after founding of Polish VHL

Registry. Patients in this group were diagnosed by "active" searching that included pedigree/clinical assessment and molecular DNA analysis in families of 106 patients with CNS haemangioblastoma, 8 patients with "angiomatosis retinae", 76 patients with RCC with clinical features characteristic of VHL (multifocal, cystic, bilateral or in patients 45 year old or younger), 15 pheochromocytoma patients.

Surveillance: In all patients from group II surveillance program was proposed. 35 of the patients decided to participate in the program in our center. Such a program was proposed only in 3 of 19 patients from group I but in the group the program did not comprise other family members and did not comprise all of systems at risk.

Lesions diagnosed: In group I: 2 asymptomatic and 16 symptomatic CNS tumors, 13 asymptomatic and 20 symptomatic retinal lesions, 4 asymptomatic and 4 symptomatic renal solid tumors and 2 symptomatic pheochromocytoma were diagnosed.

In group II 21 asymptomatic and 2 symptomatic CNS tumors, 76 asymptomatic and 2 symptomatic retinal lesions, 15 asymptomatic and 6 symptomatic renal solid tumors, 3 ELSTs were diagnosed.

Genetic counseling: In group II all VHL patients were informed about inheritance and the need of informing other family members about potential predisposition. Only 6 patients from group I received information about inheritance and none was informed about need to inform other family members about potential predisposition.

Ethical, Legal, and Social Issues

VHL and Legal Issues: Dealing with Health Insurance and Advance Treatment Directives

Thomas D. Rodenberg, Esq.

Jeter, Rains & Byrn, Blue Springs, Missouri

Mr. Rodenberg will discuss health insurance issues as they relate specifically to genetic health conditions. The focus of the discussion will be on dealing with insurance claims for those with genetic conditions. He will cover principles of contract law which impact on policy claims, policy provisions which often come into play with genetic conditions, legal issues which he has faced in representing people with VHL and practical thoughts on dealing with insurance claims. Mr. Rodenberg will also discuss legal issues relating to advance treatment directives such as living wills, health care treatment directives and powers of attorney. He will cover the legal basis for these health care documents, what each document is meant to accomplish in terms of health care decisions, how the treatment directives relate to one another, how they differ and which treatment directives should be considered by persons with VHL.

See Appendix B for Handouts from Mr. Rodenberg's session.

Putting the House in Order

Patricia Rasmussen, B.S.W., L.P.N.

Crosslake, Minnesota

Health care Directives -- how do they work? Who needs them, and why? Where do I find the forms? Do I need a lawyer? With enormous leaps in healthcare, complicated situations will arise more often and one needs to be

prepared. The health care directive can cover a few of the options, but not all them. It is recommended that health care directives have durable power of attorney for health care and be reviewed every five years, but with the way medicine is changing at present every other year would be best. Managing health care for your family needs to be thought of in advance and as it arises. Decisions on appropriate health care, under various situations, with agreed upon health care directives, should be documented to verify those decisions. The purpose of the documents is so that at any time or place those decisions will be complied with, whether or not others completely agree with them. One should make sure the facility and the doctor are willing to follow the health care directives chosen.

There are six elements required by law and they are: that the document must be in writing, dated, have the name of person, done by the person who it is for, verified by a notary or two witnesses (not from the facility a patient resides in), and has health care instructions or a health care power of attorney, or both. The six required elements allow for a variety of forms to meet special needs and handle complex issues, when one is unable to make decisions for oneself. The durable power of attorney can be broad-based and gives more flexibility in regards to choices of care. Medicine can change so fast, and a situation can be viewed differently, on a daily basis, allowing for a much different quality of survival than what is available at present. Thus the importance of a durable power of attorney for health care that appoints someone you trust to make decisions for you. This allows for updated health care choices as advances in medicine increase.

DNA Testing

DNA Testing Methods and Applications

Catherine A. Stolle, Ph.D.

Managing Director, Genetic Diagnostic Laboratory, Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

An introduction to the terminology of DNA testing, and how the various techniques are used in detecting mutations along the VHL gene. The importance of involving a genetics professional, to help in explaining the meaning of the test results.

Psychosocial, Ethical, and Legal Issues in Genetic Testing for von Hippel-Lindau Disease

Vicki Couch

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Von Hippel-Lindau disease (VHL) is a genetic disorder which causes a predisposition to specific benign tumors (angiomas), cysts, and malignant tumors in various parts of the body including the brain, retina, kidney, spinal cord, testes, pancreas, and adrenal gland. The condition follows a dominant pattern of inheritance in families. This means that each child of a person with VHL has a 50% (1 out of 2) chance of inheriting the VHL-causing gene mutation. Genetic testing can determine who in a family has inherited the predisposing gene change and thus is at risk to develop the associated tumors and cysts. Identifying at-risk relatives, who are then advised to follow specific screening recommendations, increases the likelihood of detecting tumors and cysts at an earlier, more treatable, stage, which can reduce the complications that are associated with VHL. Furthermore, gene testing has proven to be cost effective, since relatives who do not carry the VHL mutation that is running in their family do not need undergo the expensive screening tests.

Genetic counseling and genetic testing are much more complex than is generally realized. Determining whether or not one has the VHL gene is more than a "simple blood test." It is a complicated decision-making process, deserving individual attention and thought. This talk will focus on issues surrounding gene testing for individuals who might have the gene for VHL.

Prior to having their blood drawn, individuals have numerous issues to consider regarding the impact the test results will have on their employment, insurance, family relationships, self-image, and long-term plans. We will review the psychosocial, ethical, and legal implications of genetic testing for von Hippel-Lindau disease by

addressing questions commonly asked by patients seeking genetic diagnosis for themselves and their family members.

Genetic Discrimination: How much do we know and what are we doing about it?

Middelton, LA

Urologic Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

There is increasing societal perception that employers and insurance companies use personal genetic information to deny, or limit access to, employment and / or health and life insurance. Since the mid 1990's extensive state and some Federal legislative activities have responded to the public concern in an attempt to control the use of genetic information. Several studies suggest employment and insurance discrimination is a legitimate threat citing numerous examples. Other studies suggest public fear of discrimination is greater than reality. Data from several relevant studies and an overview of legislative efforts will be presented.

Optimal Clinical Care

Clinical Evaluation and Molecular Genetic Diagnosis of VHL: Susceptibility to Tumors of Kidney, Brain, Spinal Cord, Pancreas, Adrenal Gland, Eye and Inner Ear.

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Early onset clear cell renal cell carcinoma, brain and spinal cord hemangioblastomas, pancreatic neuroendocrine tumors, pheochromocytomas, retinal angiomas, and endolymphatic sac (inner ear) tumors in von Hippel-Lindau disease (VHL) [MIM 193300] occur among family members with a germline mutation of their *VHL* tumor suppressor gene on chromosome 3p25-26. Improved methodologies permit identification of the unique germline mutation in each family with VHL. Each family's unique mutation is a genetic marker for identifying family members at risk who will benefit by clinical screening and management throughout their lifetime. Our protocol for clinical screening of both symptomatic and asymptomatic at-risk family members includes: family and individual medical history, physical-neurologic examination, MRIs of brain and spine, CT of kidney, pancreas and adrenals, ultrasound of abdomen in children with addition of MRI or CT as needed, ophthalmoscopy, neurotology, ultrasound of scrotum in males, and plasma and 24-hour urinary catecholamines, and metanephrines, and urinary VMA. Apparently asymptomatic family members who were positive for *VHL* mutations had detectable VHL tumors in most cases. Recent increase in genetic testing has identified a few elderly asymptomatic family members with VHL germline mutations and no VHL tumors on screening. In these individuals, the mutation is said to be incompletely penetrant. This may be due to lack of the necessary "second hit," or to affects of modifying genes. Studies of factors contributing to incomplete penetrance are important and may lead to better risk assessment, counseling, and clinical management, prevention and control strategies.

Clinical Evaluation and Molecular Genetics of VHL: Abstract 3-19-00, GMG

Molecular diagnosis of von Hippel-Lindau disease in Brazilian Families

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Introduction: Von Hippel-Lindau (VHL) is an autosomal dominant disorder in which affected individuals are predisposed to develop a variety of neoplasms in multiple target organs, including retinal angiomas, hemangioblastomas of the central nervous system, renal cell carcinoma, pheochromocytoma, and cysts of the kidneys, pancreas, and epididymis. The basis of the disease is the presence of germ-line mutation in the *VHL* gene. The molecular diagnosis of VHL has been possible through molecular biology techniques, which allow the screening of patients and asymptomatic family members, as well the genetic counseling of these individuals.

Material and Methods: 15 VHL families were included in the study for mutation detection. DNA was extracted from peripheral lymphocytes of blood samples of each patient, following informed consent and genetic counseling. The 3 exons of *VHL* gene were amplified by PCR, and the products were sequenced for point mutation detection. Southern-blot was used for detection of large deletions of the *VHL* gene.

Results: The mutations found on the *VHL* gene in these families were analyzed and characterized, and a genotype-phenotype correlation was made in order to associate the mutations with the clinical profiles presented. New mutations were identified in Brazilian population.

Conclusion: Molecular diagnosis of VHL confirmed the suspected clinical cases, permitted the screening of asymptomatic family members and the follow up of mutation carriers.

Characteristic features in the VHL disease from a survey to urologists and neurosurgeons in Japan

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Von Hippel-Lindau (VHL) disease is one of the autosomal dominantly inherited multi-tumor syndromes. Information about this disease, including the numbers of family, incidences, and characteristics of developing tumors in this disease is not clarified yet in Japan.

To obtain information in this disease, we sent a questionnaire to neurosurgeons at 315 major hospitals and urologists at 889 hospitals in Japan in order to clarify special characteristics in this disease. The following results are observed.

1. The newly detected families at the neurosurgery division was 68, those at urology was 33. The numbers of patients in these families are 92 and 148 at neurosurgery and urology division, respectively.
2. The frequency of retinal hemangioblastoma is low in Japan compared with that in European studies (33 % in neurosurgery, 22 % in urology in Japan vs. 59 % in United Kingdom studies). The frequency of spinal cord hemangioblastoma is high (37 % in neurosurgery in Japan vs. 13 % in United Kingdom).
3. VHL disease develops tumors in the central nervous system, the retina, and the kidney. However, VHL disease in Japan also develops various types of tumors in other organs in Japan. The representative tumors are pituitary tumors, hemangioblastomas in the skin, and meningiomas.

These findings suggest that different disease conditions are present in Japan compared with those in European countries. We understand this result and try to set up a better registration system. It is important to organize a good treatment and follow up system for VHL disease in Japan.

Screening for VHL in patients with CNS Hemangioblastoma

Sven Gläsker

University of Freiburg, Germany

Objectives: Hemangioblastoma of the Central Nervous System (CNS) occurs as a sporadic entity and as a manifestation of the autosomal dominant von Hippel-Lindau disease (VHL) with the major additional components retinal angioma, renal cancer and pheochromocytoma. Since identification of the *VHL* tumor suppressor gene, genetic testing for germline mutations predisposing to VHL is available. The impact of this testing was evaluated in patients with hemangioblastomas seen in this center.

Methods: A register and database of patients with symptomatic hemangioblastomas for the last 15 years was evaluated. The *VHL* gene was analysed by SSCP method of all exons and Southern blotting for mutations and deletions of the gene.

Results: Registered are 141 patients with hemangioblastoma of the CNS. In 81 patients (57 %) there was a disease predisposing germline mutation including 8 novel mutations. Population related calculation of patients from the administrative district of Freiburg revealed *VHL* germline mutations in 22 % of the patients with hemangioblastoma. Analysis of mutation carriers for clinical information suggestive for the syndrome revealed (i) a positive family history of a brain tumor in 50 %, (ii) a history of the patient for extracranial manifestations in 36 % (retinal angioma 30 %, pheochromocytoma 6 %), and (iii) 19 % presenting with multiple brain tumors when first admitted. By genetic testing of hemangioblastoma patients without any hints for the VHL disease we identified mutation carriers in 14 %. Sensitivity of *VHL* germline testing was 86 %.

Conclusions: DNA analysis for *VHL* germline mutations is clearly superior to clinical information in diagnosis of VHL. Although the percentage of VHL associated hemangioblastoma decreases after the fourth decade of life and is infrequent in patients without other symptomatic lesions and a negative family history, it is recommended that every patient with CNS hemangioblastoma should be screened for VHL germline mutations. This provides the key information and enables screening for extra-neurological tumors of the patients and investigations of the patient's family to ameliorate management of von Hippel-Lindau disease.

VHL in Italy

Giuseppe Opocher, Alessandra Murgia, Maddalena Martella, Stefano Piermarocchi, Giuseppe Lo Giudice, Carla Scaroni, Roberto Ragazzi, Tommaso Prayer-Galletti, Paolo Bernante, Pietro Zucchetta, Eva Orzan, Marina Gardiman, Roberto Faggini, Carla Carollo, Giorgio Perilongo.

"VHL Padova network" University of Padua, Padua Italy.

The efficient clinical management of patients affected by multisystem diseases, such as the von Hippel-Lindau (VHL) disease, requires a multidisciplinary approach and the work of a coordinated team.

With the aim of improving early detection and the quality of clinical care for VHL patients, we have established in Padua, in 1997, a collaborative VHL group with the participation of several clinical units (endocrinology, ophthalmology, urology, neurosurgery, neuroradiology, pathology, audiology and nephrology), and with the support of the molecular genetics laboratory.

Twenty new cases of VHL disease have been so far detected.

The strategy adopted for the molecular analysis has allowed us to detect the disease-causing mutations in 94% of the VHL patients tested.

The integrated clinical approach improves the quality of health care offered to these patients and to their families, providing a facilitated access to different clinical specialists and a coordinated follow-up protocol.

We are planning to extend our experience of organized VHL clinical team to other centers in Italy. We have already established collaborations with several peripheral hospitals that can represent satellite centers for our VHL team in the north east of Italy.

Results of VHL gene mutation analysis in the Netherlands

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Objective To summarise the results of mutation analysis of the VHL gene in familial and sporadic cases of VHL disease diagnosed in the Netherlands.

Patients and methods Familial (n=25) and sporadic (n=7) VHL patients, and additionally, two sporadic patients with VHL-related tumours, not fulfilling current diagnostic criteria for VHL disease, were investigated by direct sequencing of the coding region, quantitative Southern blot and FISH analysis of the VHL gene.

Results We report 34 VHL germline mutations, including eight novel germline mutations in the open reading frame of the VHL gene. Analyses of genotype-phenotype correlations revealed that carriers of deletions and mutations predicting a truncated protein are associated with a low risk for pheochromocytoma but, with a preponderance of cerebellar haemangioblastoma. We identified four VHL germline mutations as definitely *de novo* in nine sporadic patients. The family history of three further sporadic VHL patients was suggestive for *de novo* occurrence of the mutations. One patient was adopted as a child. One of the nine patients shared a VHL germline mutation (P81S) with a clinically unaffected parent. The absence of VHL-related manifestations in three elder carriers (P81S, aged 64 and 77 years; R64P aged 55 years) suggested non-penetrance of VHL disease.

Conclusions In our series *de novo* VHL mutations occur in a 12% (4/34) to 21% (7/34) of identified independent cases of VHL disease. Germline mutations are found in patients not fulfilling the currently accepted diagnostic criteria. Non-penetrance of two particular VHL germline mutations was demonstrated and has implications for genetic counselling.

Preserving Organ Function: Adrenals

Plasma Free Metanephrines: A Promising new Neuro-chemical test for Pheochromocytoma in VHL Patients

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Objective: In this study we evaluated the diagnostic value of plasma free metanephrines for the diagnosis of hereditary pheochromocytoma (HP) and sporadic (SP) and extending our previous published results (Ann Int Med 1995;123:101-109 and NEJM 1999, 340:1872-9).

Design and Methods: Plasma free metanephrines (Mets), catecholamines (Cats) and urinary metanephrines (Umets) were measured in 120 patients with a histological proven pheochromocytoma: 81 patients had a SP and 39 had a HP (30 VHL and 9 MEN-2 patients). A group of 164 patients in which a pheochromocytoma was excluded by CT-scan or MRI served as a control group.

Results: In HP, only 1 patient with VHL out of 39 subjects had false-negative plasma Mets (sensitivity 97%) while 12 patients had false-negative plasma Cats (sensitivity 69%). All 81 patients with a SP had elevated plasma Mets (sensitivity 100%) while plasma Cats were false-negative in 5 out of 81 patients (sensitivity 94%). The specificities of all compounds were higher in the HP patients than in the SP's. In the HP patients the specificities were higher for plasma Mets (92%) and Umets (96%) as compared to plasma Cats (77%).

	Positive predictive value (%)			Negative predictive value (%)		
	Plasma Cats	Plasma Mets	Umets	Plasma Cats	Plasma Mets	Umets
HP	59	84	89	84	99	83
SP	67	72	83	90	100	88

Conclusions: Plasma Mets are superior to plasma Cats or Umets for exclusion of a pheochromocytoma since their negative predictive value is 99-100%. In addition, plasma Mets and Umets are also superior for confirming a pheochromocytoma.

Surgery for Adrenal Tumors in VHL: Traditional and Laparoscopic Approaches

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Laparoscopic adrenalectomy has become our procedure of choice for most benign, functioning and nonfunctioning adrenal tumors, measuring less than 10 cm. Nearly 150 such procedures have been performed at the Mayo Clinic since 1994. Bilateral procedures and partial adrenalectomies can be performed utilizing this technology, making this approach a viable and practical option for patients with VHL and pheochromocytomas. Laparoscopic procedures, although technically more demanding, are associated with lower overall morbidity, less pain, and faster recovery than their traditional open counterparts. Multiple prior abdominal operations and its associated scarring, as well as the need for concomitant renal and/or pancreatic procedures in VHL patients, may however preclude a laparoscopic approach in favor of the more traditional posterior or anterior open operations.

Intraoperative Imaging

Exploring the Benefits of High-Field Strength Intraoperative Magnetic Resonance Imaging

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Achieving a Diagnosis

Strategies for finding the undiagnosed patient with VHL

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Three independent studies revealed a prevalence of 1:40000 to 1:50000 for VHL. Thus, 2000 VHL patients can be estimated in Germany

Strategies to identify VHL patients have three branches; retrospective evaluation of patients at risk, prospective studies for such patients and improvement of structures in a clinical VHL center. We present results based on such innovations from the recent years.

1. Information events have been offered and performed regularly twice a year including regional meetings and teaching courses for all German speaking countries including Switzerland Austria, Northern Tyrolia and

Luxemburg. Internet informations on VHL have been installed. Colleagues have been trained by local or congress contributions.

2. We installed a VHL center for Germany which includes genetic testing, clinical counselling, clinical screening, follow up investigations and laser or surgical treatment of all VHL associated lesions. We developed new facilities and are the first introducing laparoscopic adrenal sparing surgery for pheochromocytoma.
3. We have systematically addressed pheochromocytoma as a feature of VHL to all 300 German pediatric departments. Based on a 95% response rate we established a German childhood and youth pheochromocytoma registry representing the last 2.5 decades during which 80% of the registered patients have undergone DNA testing.
4. Prospective programs have been initiated for retinal angiomas in Berlin and Essen and for all VHL-associated lesions in southwestern Germany.

The number of identified patients in Germany has now risen to 356, but this is only 18% of the estimated number. The prevalence of VHL in Germany may be overestimated.

The conclusion of all our efforts is that prospective nationwide programs are the most relevant way to identify VHL patients. Although better treatment and avoiding unnecessary investigations and inappropriate operations results in decreased morbidity and mortality public health support is still insufficient.

Improving Outcomes in VHL Families

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A clinical screening program for von Hippel-Lindau disease (VHL) in Newfoundland was developed in 1982 following referral of members of a large VHL family to the Ocular Genetics clinic. This screening program resulted in earlier diagnosis and more successful treatment of tumours with fewer deaths and disabilities in this VHL family, and in other VHL families identified later. With this successful treatment, the natural history of VHL has changed. Patients (N=45) have had more tumours than previously, including more frequent renal cell carcinomas (37%), and cerebellar (35%) or spinal cord (21%) hemangioblastomas. Retinal angiomas (72%) and pheochromocytomas (77%) remain the most frequent tumours. The clinical screening program has been revised to reflect this pattern of disease, and as new technology becomes available. A second VHL family has a similar clinical phenotype with frequent pheochromocytomas. Three smaller families have had no pheochromocytomas but more frequent cerebellar and spinal cord hemangioblastomas.

Genetic testing is now available for 5 of 7 definite VHL families, and there has been > 90% uptake of genetic testing for those at risk. A mutation has not been identified in two families with VHL, or in 2 other families with renal cell carcinoma only. Although patients are living longer and with fewer long-term disabilities, they do require psychosocial, emotional and financial support to handle the stresses of this life-long disorder.

Preserving Organ Function: Kidney and Pancreas

Organ Sparing Surgery: Kidney Cancer, Adrenal Tumors

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Patients with hereditary forms of renal cancer and pheochromocytoma are predisposed to develop new tumors over the course of their life. The goal of organ sparing surgery is to maintain quality of life by preservation of normal organ function. Surgical removal of tumors in patients with hereditary tumors varies by country and institution. Our group has practiced following renal and adrenal tumors before recommending surgery. Renal cancers are followed until the largest renal tumor reaches 3 cm in diameter before recommending surgery. Pheochromocytoma are followed until symptoms are evidence of function are demonstrated. No metastases

have occurred with a median of 5 years follow-up in renal cancer patients. No side effects of observing small nonfunctional pheochromocytoma have been observed.

Management of neuroendocrine tumor of the pancreas in patient with VHL disease

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The frequency of neuroendocrine tumors (NET) in VHL patients ranges between 10 and 20 percent. An isolated NET can represent the only lesion of VHL disease. Asymptomatic hormone hypersecretion, especially somatostatin, is not exceptional in VHL patients with NET. The differential diagnosis between NET and other vascular tumors of the pancreas in VHL is limited (pancreatic hemangioblastomas, pancreatic metastasis from renal cancer, vascularized serous cystadenomas in solid form). CT scan, somatostatin receptor scintigraphy and endoscopic ultrasonography (+/- needle aspiration) can help in preoperative distinction. We have shown that nesidioblastosis adjacent to NET can be encountered in this disease. NET in VHL patients carry a risk of malignant transformation, and metastatic progression has been shown to occur in about 25% of cases. Surgery can be required to treat symptomatic NET or to prevent metastatic progression in large lesions. We and others found that NET greater than 3 cm. in diameter often displayed high tumor aggressivity with invasion of the duodenum and lymph nodes or liver metastasis. Therapeutic decisions in VHL patients can be problematic as the presence of multiple NET of various sizes makes surgical resection options difficult. Moreover, these patients are often simultaneously affected by various life-threatening tumors of the central nervous system, adrenal glands or kidneys requiring priority treatment.

In conclusion, NET is the most relevant pancreatic lesion in VHL disease. Therapeutic decisions must take into account the size and number of NET and the presence of other life-threatening VHL lesions.

Nephron-sparing surgical techniques

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Several nephron-sparing (nss) surgical techniques can be used for VHL associated renal cell carcinoma. Partial nephrectomy has been the most extensively utilized. When technically feasible this should be used as an initial approach for bilateral tumors as it affords excellent patient survival. Nonetheless, there is a high local recurrence rate, and up to 25% of patients may culminate in end stage renal disease. For these patients, renal transplantation provides excellent renal function with a low risk for tumor recurrence. Newer techniques that have been applied to patients with sporadic renal cell carcinoma may be valuable for VHL patients. Noteable is laparoscopic renal cryoablation. This technique is developmental but early results suggest satisfactory short term tumor control in a well tolerated procedure.

Hemangioblastomas of the CNS and Retina

Central Nervous System von Hippel-Lindau—Management and Treatment

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Von Hippel-Lindau Syndrome (VHL) is a multi-organ disease involving several medical subspecialties from the neurosurgeon to the internist to the medical geneticist. Both familial and sporadic cases of this disease can occur and the challenge for treatment lies in early diagnosis. To this end the utilization of MRI has facilitated detection of VHL. We will present our retrospective case study comprising a decade's worth of neurosurgical experience managing this illness during the MRI era at the Mayo Clinic.

Predicting Growth of CNS Hemangioblastomas

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Hemangioblastomas in VHL are pathologically non-malignant and multicentric. At least 50 percent of patients with VHL will develop CNS hemangioblastomas, the treatment mainstay for which is surgical resection of symptomatic lesions.

We performed a retrospective review of 160 patients with CNS and spinal hemangioblastomas followed at the NIH. Preliminary data demonstrate that between 15 and 33% of patients had tumor progression over a three-year period of observation. Of these, 47% of patients had continuous growth as demonstrated on serial MRI scans, while 53% had quiescent phases between growth. It is not known what factors may predict, or influence, tumor growth.

A prospective natural history studies of CNS lesions is required to define the lesions most likely to become symptomatic (produce symptoms or cysts). Knowledge of the natural history of untreated and treated hemangioblastomas in patients with von Hippel-Lindau disease is critically needed to choose the most appropriate therapy and the optimal timing of intervention. The multicentric nature of these lesions with benign pathology makes it important that intervention is offered only to patients whose lesions produce symptoms. Other lesions can be followed expectantly.

Hemangioblastomas of the CNS and retina: impact of VHL and interferon treatment

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We studied the prevalence and impact on outcome of VHL among 110 consecutive patients operated on for HB(s) of the CNS and 36 patients treated for retinal HB(s) in Helsinki. We also assessed the efficacy of interferon-alpha-2a (IFN α 2a) in treatment of asymptomatic HBs of the CNS and retina.

All available follow-up data, clinical and radiological examination, mutation and pedigree analysis were obtained (median follow-up 14 and 10 years, respectively) with no patient lost to follow-up.

Four patients with altogether 15 HBs of the CNS and 3 of the retina, were treated with subcutaneous IFN α 2a for 12 months at 3x10⁶ IU 3x/week.

Of the 110 CNS HB patients, less than 15% had VHL with a median length of life 46 years. The growth rates of HBs were similar in VHL and non-VHL patients. The prevalence of VHL was one third to one half in patients with retinal HB(s). The appearance of HBs was similar, but the prognosis of vision was worse in VHL than sporadic patients.

No de novo HBs were detected during the IFN α 2a therapy, which may decrease blood flow in HBs as suggested by shrinkage and diminished leakage of two retinal HBs.

VHL among patients with HB(s) of the CNS and retina was less frequent than presented earlier. HB patients with visceral cysts do not necessarily have VHL. A larger study with a higher and denser IFN α 2a dosage in a combination with other antiangiogenic agents, is warranted in treatment of HBs of the CNS and retina.

Retinal hemangioblastomas in VHL disease: a case-control study

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Retinal hemangioblastomas, one of the most common manifestations of von Hippel Lindau disease, may lead to sight-threatening complications. A retrospective case control study was designed in order to characterize retinal hemangioblastomas in the French population of VHL patients.

The survey was based on 205 VHL patients. Cases were 103 patients with ocular manifestations and controls were 102 patients, presenting without retinal manifestation at inclusion, and age-and-gender-matched. A questionnaire was sent to the referred ophthalmologists in order to ascertain the cases. Ophthalmological examination was also indicated in controls to avoid misclassification. Mean follow-up period was 7 years (2-35 years). Natural history and prognosis factors were looked for using logistic regression analysis. VHL Germline mutations were studied by SSCP and Southern blotting and genotype-phenotype correlations were evaluated according to the functional domain of the VHL protein.

The clinical features and genetic mechanisms in hemangioblastoma of central nervous system

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Von-Hippel Lindau disease (VHL) is an autosomal dominant inherited disorder characterized by hemangioblastoma (HB) of the central nervous system, retinal angioma (RA), and renal cell carcinoma (RCC). The objective of this study is to compare HB associated with VHL with sporadic HB in the view of clinical features and genetic mechanisms. Forty two patients bearing HB (13 cases of VHL and 29 cases of sporadic HB) were studied with respect to age of onset, number of tumors, location, accompanying lesions, genetic mechanism, and prognosis. The mean age of onset in VHL and sporadic HB was 37.5 and 43.6 years respectively. The incidence of multiple HBs is 92% in the VHL group and 13% in the sporadic HB group. There was no obvious difference in the location of HB between the VHL and sporadic HB groups.

In the VHL group, accompanying lesions such as renal cysts, RA and pancreatic lesions, RCC, and pheochromocytoma were manifested in 60%, 50%, 40% and 16% respectively, whereas there were quite a few instances of renal and hepatic cysts in the sporadic HB group. VHL gene analysis for lymphocyte DNA of patients bearing HB revealed germ-line mutation in 63% of the VHL group but none in the sporadic HB group. However VHL gene analysis of tumor tissue showed a somatic mutation in 34% of the sporadic HB group. Mortality rate was higher in the VHL group(15%) than in the sporadic HB group (6%). Patients in both the VHL and sporadic groups showed more than 80% function on the Karnofski Performance Scale (KPS) except for those with RCC and cervical cord lesions. Nowadays VHL can be diagnosed with gene analysis even before the clinical onset. Therefore further consideration of the ethical problems is desirable, including the hiring of a professional counselor.

Stereotactic Radiosurgery for Hemangioblastoma

Stereotactic Radiosurgery for Patients with von Hippel-Lindau Disease

Bruce E. Pollock, MD

Mayo Clinic and Foundation, Rochester, MN USA

Posterior fossa hemangioblastomas are one of the leading causes of death for patients with von Hippel-Lindau Disease (VHL). Although histologically benign, they can behave aggressively due to their location and their tendency to recur after operative resection. Theoretically, hemangioblastomas are ideal cases for radiosurgery based on their imaging characteristics, vascular histology, and non-infiltrative nature. Published studies to date for hemangioblastoma radiosurgery has shown approximately a 75% to 85% local control rate for individual tumors at follow-up intervals of 5 years. It appears that higher radiation doses correlate with improved tumor

control. Based on our experience, we feel that radiosurgery is safe and effective for VHL patients with small to moderate sized hemangioblastomas, either as a primary treatment or for recurrent tumors after prior resection. Radiosurgery is not appropriate for large cystic tumors.

Sunday Meditation

Keeping your Balance, Going the Distance.

Jay Platt, Gunnery Sergeant, United States Marine Corps, retired
Jay Platt International, Cartersville, Georgia

What's your reaction when you're stuck in traffic...on hold forever...or faced with others' thoughtless or insensitive behavior? What about life's more serious issues like surgery, death and divorce? Would you like a better way of dealing with these stresses and more? Then join speaker, author, and management consultant, Jay Platt as he shares some of the lessons he's learned about life and living from his new book, *A Time to Walk: Life Lessons Learned on the Appalachian Trail*.

Tumor Localization and Assessment

Tumor Assessment using Imaging Techniques.

Peter L. Choyke

Despite the dramatic advances in molecular biology, imaging remains the only reliable method of detecting and following the tumors associated with von Hippel Lindau syndrome. Fortunately, a number of technical advances have been made that have improved the quality and value of imaging. Helical multidetector CT scanners now permit extremely rapid surveys of the body. New MRI units with high strength gradients allow thin, high resolution sections to be performed in much shorter times than was previously possible. Contrast agents for ultrasound are now in the FDA approval process and may make sonography a much more useful screening tool for VHL. Positron emission tomography (PET scanning) is being used more routinely in the evaluation of tumors because it provides unique information on metabolic activity. Image analysis is improving to the extent that volumetric data may be routinely obtained from 3D data sets. Of major importance to patients is that their entire imaging portfolio can now be electronically stored (and transmitted).

Imaging is also being used to guide therapy, for example with Radiofrequency ablation of renal tumors. Research is ongoing into the development of contrast agents with selectivity for particular tumors. The progressive improvements in imaging technology will be of great benefit to patients with von Hippel Lindau.

Radioactive Plaque Therapy for Large Eye Tumors

Radioactive plaque therapy of von Hippel tumors

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Purpose: To describe the indications for eye plaque radiotherapy of von Hippel tumors, the physical and chemical structure of radioactive eye plaques used in this therapy, the typical radiation target doses and dose-distributions associated with eye plaque radiotherapy of von Hippel tumors, the expected post-treatment responses of the retinal tumors and other intraocular tissues, and the potential risks and complications of such treatment.

Methods: Review of personal experience and reported series of cases.

Results: Selected illustrative cases of von Hippel tumors treated by plaque radiotherapy showing clinical regression of the lesions.

Conclusions: Radioactive eye plaque therapy can be used to treat selected von Hippel tumors. Lesions most amenable to such therapy are larger tumors (tumor diameter >4.5 mm) located more than 3 mm from the optic disc. Plaque radiotherapy is not applicable to eyes with juxtapapillary and epipapillary von Hippel tumors.

Ruthenium brachytherapy of retinal angioma

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Augenlinik, Klinikum Benjamin Franklin der Freien Universität, Berlin, Germany

Purpose: To evaluate the efficacy and safety of ruthenium-106 brachytherapy of large peripheral retinal capillary hemangiomas.

Patients and Methods: In 25 eyes of 24 patients peripheral capillary retinal hemangiomas were treated by brachytherapy using 106-ruthenium/106-rhodium plaques. Eyes were reviewed for hemangioma regression after brachytherapy, occurrence of retinal detachment, requirement of additional vitreoretinal surgery, final visual outcome and final retinal status.

Results: Preoperative mean visual acuity of all eyes treated was 20/60, mean hemangioma diameter was 3.8 mm, corresponding to approx. 2 disc diameter. In 14 eyes the retina was attached preoperatively, 8 eyes showed an exudative detachment and 3 eyes showed a traction detachment. 15 patients had definite von Hippel-Lindau syndrome. 23 of 25 hemangiomas could be destroyed by single brachytherapy. In 16 eyes a favourable outcome could be achieved. In 9 eyes outcome was unfavorable, characterized by a severe drop in visual acuity, a persisting exudative retinal detachment or a recurrent traction detachment. In one eye requiring repeated brachytherapy irradiation retinopathy occurred. Hemangiomas up to a size of approx. 5.0 mm without preoperative exudative detachment could be treated safely by brachytherapy, whereas a larger hemangioma size or a preexisting exudative retinal detachment predisposed to an unfavourable outcome.

Conclusions: Solitary peripheral retinal hemangioma can be ablated effectively by ruthenium-106 brachytherapy. A favourable outcome can be expected if the hemangioma diameter is 5.0 mm or smaller and if there is no preoperative exudative retinal detachment.

Innovative Eye Treatments and Clinical Outcomes

Management of the Retinal Tumors in von Hippel-Lindau (VHL) Disease

Helmut Buettner, Dennis M. Robertson

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A practical approach to the management of the retinal tumors in VHL-disease will be presented. The natural course, role of observation and the preferred technique of photocoagulation will be discussed. Indications for cryotherapy and scleral buckling as well as the less frequently indicated modalities of vitrectomy, transpupillary thermotherapy (TTT), brachytherapy and external beam radiation will be included.

Innovative Eye Treatments and Clinical Outcomes

Emily Y. Chew, M.D.

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Retinal and cerebellar hemangioblastoma are the most common and frequently the earliest manifestations of von Hippel Lindau (VHL) disease. As many as 60% of patients in some studies of large kindreds may have ocular involvement, namely retinal angiomas.^{1,2,3,4,5,6,7} The clinical appearance of these angiomas is very typical and diagnostic of VHL. The initial appearance of a retinal angioma is a subtle red or grayish dot no larger than a few hundred microns. As the proliferation of the vascular tumors (mostly of capillaries) progresses, secondary alterations occur to produce a distinctive clinical appearance. The blood vessels leading to and away from the tumor become characteristically dilated with marked enlargement. This tumor can lead to leakage of fluid and fatty deposits both around the tumor and in the central important area of the retina, the macula, which is responsible for the fine vision needed for reading, driving, etc. If the angiomas enlarge to an extent that the retina

can be detached, hemorrhaging and scarring can occur. These can all lead to decrease in visual acuity of the affected individual. Rarely can these tumors regress spontaneously.⁸

The usual treatment of the retinal angiomas will depend on the location and size of the lesions. Small lesions are easy to treat successfully while large lesions are notoriously difficult to treat. Photocoagulation with argon laser can eradicate small retinal angiomas in most locations.^{9,10} However, for those tumors too large or located in the very periphery of the retina, cryotherapy (freezing treatment) may be indicated. If the tumor is located on the optic nerve, the nerve that connects the eye to the brain, treatment is fraught with difficulties. Marked adverse side-effects are associated with treatment of such tumors with laser photocoagulation. Fortunately, these tumors may remain asymptomatic for long periods of time. For patients with the more severe changes such as retinal detachment, hemorrhage and scarring, the procedure called vitrectomy can be performed.¹¹ This involves the introduction of microinstruments under the guidance of a microscope to remove the areas of scarring and to flatten out the retina. Other treatments that have had some limited success include radiotherapy. However, experience with this modality is somewhat limited. Further studies will be required.

Although many of these eye lesions can be treated successfully with some of the usual treatments, the patients with the more aggressive tumors, especially those on the optic nerve have a poor visual result. Newer treatments must be developed to treat lesions that eventually lead to severe vision loss or even enucleation or removal of the eye.

Using tissue microdissection and polymerase chain reaction amplification, investigators at the National Eye Institute/National Institutes of Health have analyzed seven retinal angiomas associated with VHL for genetic abnormalities found within the eye lesions.¹² Similar to results of studies of kidney cancer and cerebellar hemangioblastoma, the VHL patients inherit a mutated copy of the VHL gene (inactivated in the constitutional cells) and at the molecular level of the tumor; the second copy of the gene is also inactivated; so at the molecular level, VHL disease is an autosomal recessive mutation. The product of the VHL gene, pVHL, is important in a feedback system that regulates the production of Vascular Endothelial Growth Factor (VEGF). VEGF is important in promoting the growth of new vessels that become part of the eye disease. VEGF, also known as Vascular Permeability Factor (VPF), is the growth factor, that exhibits vascular permeability-inducing activity or causing these blood vessels to “leak” important constituents of the blood. Previous studies have confirmed that overproduction of VEGF is a hallmark of renal cell carcinomas and hemangioblastomas.^{13,14} Similar findings of elevated VEGF are found in retinal angiomas.¹²

These findings are both interesting and clinically important for the rationale for other experimental treatments, which may be efficacious in treating the eye as well as the systemic tumors. A number of compounds are being tested in various phases of clinical trials for a number of eye diseases such as diabetic retinopathy and age-related macular degeneration. These diseases also have demonstrated an abundance of VEGF. These trials are currently being conducted with no results yet available. There are also other compounds that are potent inhibitors of VEGF and they will be studied in clinical trials for both ocular and systemic disease. Such protocols are currently being developed at the NIH.

An update of such protocols and other forms of treatment will be discussed at the meeting.

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Ophthalmic screening guidelines for VHL and fluorescein-potentiated argon laser treatment of retinal hemangiomas

Michael B. Gorin

University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Fifty five angiomas in 25 eyes of 17 individuals were treated with one or more sessions of fluorescein potentiated argon laser, FPAL, therapy. Lesions ranged in size from 200 to 4000 micron in diameter. 96% of lesions demonstrated complete closure with fluorescein angiography/angiography within 5 sessions and all tumors underwent almost complete regression and feeder vessel attenuation within eight sessions.

Lesions were responsive to therapy even in the presence of significant subretinal fluid or serous detachment. The combination of fluorescein with argon laser treatment increased the selectivity of treatment, lowered laser energy requirements and allowed the efficient absorption of light energy in lesions that are poorly absorptive or reflective to blue-green or yellow-wavelength light.

Long-term follow-up of retinal angiomatosis and genotype-phenotype correlation

Michael H. Foerster, Klaus-M. Kreuzel, Hartmut P.H. Neumann, Thomas Heinichen

Augenlinik, Klinikum Benjamin Franklin der Freien Universität, Hindenburgdamm 30, D-12200 Berlin, Germany

Purpose: To evaluate the clinical course of retinal angiomatosis in von Hippel-Lindau disease (VHL) and to determine any correlation between retinal angioma number, VHL disease severity and genotype.

Patients and Methods: In 55 VHL patients presenting with retinal angiomatosis number and size of angiomas, visual function and treatment outcome was evaluated. In addition a work-up for other VHL-lesions and a molecular genetic test for the causative mutation was performed.

Results: Mean follow-up was 5.8 years. The calculated prevalence for bilateral retinal angiomatosis was 100% at age 56.4 years. The prevalence of legal blindness due to retinal angiomatosis was calculated to 41% of all eyes at age 61.1 years. Legal blindness occurred at a mean age of 23.2 years: risk factors included large angiomas, manifestation at a younger age or symptomatic angiomatosis. Analysis of growth behavior showed that large angiomas which become symptomatic in young adults, start growing in childhood. In uncomplicated angiomatosis, development of new angiomas was slow and only small angiomas were detected on regular follow-up. Larger angiomas after short follow-up intervals however were observed in eyes showing multiple retinal angiomas or retinal detachment. The number of retinal angiomas per patients showed a positive correlation to the number of other organs affected by VHL disease. Patients carrying a missense mutation developed a smaller number of angiomas.

Conclusions: Retinal angiomas in VHL disease bears a high risk for severe vision loss at a young age. A lifelong ocular screening for presymptomatic lesions starting at pre-school age therefore is essential after determination of the gene-carrier status. Severity of retinal angiomas shows a positive correlation to severity of VHL disease and to the type of the causative mutation.

Progress in Research

Capsule Summary of the Thursday Research Meeting

Ann Killary, Ph.D.

M.D. Anderson Cancer Research Center, Houston, Texas

Anti-angiogenic therapy for VHL syndrome

Adrian L Harris, ICRF

Medical Oncology Unit, The Churchill, Oxford Radcliffe Hospital Oxford, U.K.

Mutations in VHL result in up regulation of the hypoxia inducible factor signalling pathway. This pathway regulates many genes that are regulated in normal tissues by hypoxia, that become consistently activated as a result of the VHL mutation. Amongst these genes are vascular endothelial growth factor, which is a key player in tumour angiogenesis, the development of new blood vessels. It is also important in normal tissue function.

Because VEGF regulated by hypoxia is important in tumour growth and metastasis, many drugs are being developed to block the VEGF function. VEGF is a logical target in VHL to try and cause regressions or prevent development of CNS, renal and retinal lesions but long term drug use may be necessary. In many cases for clinical trials in cancer, drugs have only being given for three months and therefore long term toxicities are unknown. In addition in cancer blood vessels are newly formed although in VHL they have often being present for many years. This may effect their ability to respond to VEGF therapy.

In addition as we gain more knowledge on angiogenic pathways, we find there are other angiogenic factors such as endothelium 1 and platelet growth factor B and adrenomedullin that are angiogenic and also regulated by HIF. Nevertheless it is worthwhile analysing the effects of VEGF inhibitors for their ability to control symptoms and disease in VHL. The types of lesions that are most likely to benefit would be those associated with vessel leakiness such as retinal oedema, cerebral oedema, spinal cord oedema around haemangioblastomas.

There is a clinical trial running internationally using SU5416 to block the VEGF signalling pathways. We initially have treated 3 patients for a minimum duration of 3 months each to assess ability to cause regression of, a range of lesions including retinal, CNS and renal. In no cases so far have we seen objective regressions although the disease has been stable over this time period. In addition we are attempting to analyse oedema around lesions which might be reduced by inhibition of VEGF, using MRI. From our studies in cancer it is clear that blood vessels vary substantially in the amount of VEGF binding to each individual vessel and this may effect the behaviour of disease and its response to therapy. If this variability exists in VHL or vessels that are matured it may be much harder to cause regression although prevention may be a better strategy. Also anti-angiogenic drugs with a broader spectrum of activity should be analysed based on the multiple pathways that could be involved.

Symposium Consensus Meeting

Reaching Consensus on Key Issues from this Symposium.

Thanks and Farewell

Forthcoming Meetings

Fifth VHL Symposium, Padua, Italy, 2002

Co-sponsored with the University of Padua

Chairman: Giuseppe Opocher, Genetics

U.S. Annual Meeting Palo Alto, California, June 22-24, 2001

Co-sponsored with Stanford University Medical Center

Chairman: John R. Adler, Neurosurgery

Speaker Biographies

Augsburger, James J., M.D. Ophthalmology, University of Cincinnati College of Medicine, Cincinnati, Ohio. Dr. Augsburger is a graduate of Heidelberg College (BS, 1970) and the University of Cincinnati College of Medicine (MD, 1974). Following medical school, he completed a one-year internship (mixed medical) at the Cleveland Clinic (1974-5) and a three-year ophthalmology residency at the University of Cincinnati (1975-8). He then served a two-year fellowship in vitreoretinal surgery and ocular oncology at Wills Eye Hospital in Philadelphia, Pennsylvania (1978-80). He joined the staff of Wills Eye Hospital as a specialist in ocular oncology in 1980 and remained at that institution until his appointment at the University of Cincinnati in 1999. He is the author of over 200 peer-reviewed articles and over 50 book chapters on various aspects of ocular tumors.

Brauch, Hiltrud, Ph.D. Associate Professor, Fischer-Bosch Institut, Stuttgart, Germany. Dr. Brauch received her Ph.D. from the Institute of Immunology, University of Heidelberg. She spent four years at the U.S. National Cancer Institute working under Dr. Zbar. She was a Section Head at the Laboratory of Molecular Pathology at the Technical University of Munich 1992-96, and Associate Professor and Section Head of the Research Laboratory and Laboratory of Diagnostics in Gynecology and Obstetrics, Women's Hospital Eppendorf, Medical School, University of Hamburg 1996-99. She has recently joined the Fischer-Bosch Institute as Associate Professor. She was part of the team that found the VHL gene in 1993. Her published papers include identification of link between carcinogen exposure and VHL gene damage and RCC, and identification of an association between somatic VHL mutations and advanced tumor stage in clear cell RCC. She has also studied genetic polymorphisms and expression of human xenobiotic and drug metabolizing enzymes for the elucidation of breast cancer risk and predictive factors. Her most recent article is in the Journal of the National Cancer Institute 91:854-861, 1999.

Buettner, Helmut, M.D. Professor of Ophthalmology and Consultant in the Department of Ophthalmology, Mayo Clinic, Rochester, Minnesota. Dr. Buettner is Professor of Ophthalmology and Consultant in the Department of Ophthalmology at the Mayo Clinic. He graduated from the University of Goettingen School of Medicine, in Germany, and completed his internship at municipal hospitals in Bassum, Delmenhorst, and Bad Godesberg. He then pursued internships in Vitreoretinal Diseases at Bascom Palmer Eye Institute and University of Miami School of Medicine, followed by fellowships in forensic pathology at the University of Bonn and Bascom Palmer Eye Institute, University of Miami School of Medicine. He has been with the Mayo Clinic since 1975.

Caldwell, M. Craig, Ph.D. Intramural Research Training Award (IRTA), National Institute on Aging, Baltimore, Maryland. Dr. Caldwell received his bachelor's degree from Northeast Louisiana University and his doctorate from Texas A&M University. He is currently a postdoctoral research associate with Dr. Myriam Gorospe at the Gerontology Research Center of the National Institute on Aging.

Casali da Rocha, José Cláudio, M.D. Oncology, Instituto Ludwig, Sao Paulo, Brazil. Dr. Casali is an oncologist and Ph.D. candidate, with a special interest in familial cancers, doing research in mutation detection in VHL at Ludwig Institute for Cancer Research, São Paulo

Chauveau, Dominique, M.D., Ph.D. Nephrology, Hôpital Necker, Paris. Dr. Chauveau is a member of the French VHL Study Group (GEFVHL).

Chew, Emily Y., M.D. Medical Officer, Division of Biometry & Epidemiology, National Eye Institute, National Institutes of Health, Bethesda, Maryland. Dr. Chew was previously an Assistant Professor and lecturer at the University of Toronto, Department of Ophthalmology. Dr. Chew has numerous publications, including 60 published articles and nine book chapters.

Choyke, Peter L., M.D. Chief of MRI, Diagnostic Radiology Branch, Clinical Center, National Institutes of Health, Bethesda, Maryland. Dr. Choyke trained at Yale-New Haven Hospital in Diagnostic Radiology and completed a fellowship in cross sectional imaging at the University of Pennsylvania. He joined NIH in 1987 where he has worked closely with the Urologic Oncology Branch under the direction of Dr. W. Marston Linehan. Dr. Choyke has a special interest in the imaging of von Hippel-Lindau disease along with other hereditary conditions that affect the kidneys. He is especially interested in the abdominal manifestations of VHL and the impact imaging studies have on organ-preserving treatments.

Couch, Vicki, M.S. Genetic Counselor, Department of Medical Genetics, Mayo Clinic, Rochester, Minnesota. Ms. Couch is an instructor in Medical Genetics, and is the clinic coordinator for the von Hippel-Lindau clinic at the Mayo Clinic. She also works with the Family Ascertainment Core of the Mayo Clinic Cancer Center's Familial Cancer Program. She has written several articles on VHL and on familial cancer syndromes for scientific journals.

Czyzyk-Krzeska, Maria F., M.D. Assistant Professor of Physiology, University of Cincinnati College of Medicine, Cincinnati, Ohio. Dr. Czyzyk-Krzeska earned her medical and doctoral degrees at Warsaw Medical School in Poland, studying respiratory-related discharge patterns of sympathetic nerve activity in the spontaneously hypertensive rat. She did a postdoctoral fellowship at the University of North Carolina 1991-94, and has been at the University of Cincinnati since 1994. She has published more than 20 peer-reviewed papers and many chapters and editorials, concerning aspects of oxygenation, recently concentrating on VEGF and VHL.

Davidowitz, Eliot J., Ph.D. Molecular biologist, Albert Einstein College of Medicine, Bronx, New York. Dr. Davidowitz was born in Manhattan, NY and spent his formative years in Long Beach, NY. His interest in genetics and plant biology led him to Cornell University where he attained his B.S. in biology with a concentration in genetics. After working in a molecular biology lab at Rockefeller University, he moved to Cleveland, OH to study at Case Western Reserve University where he attained his Ph.D. in molecular biology in 1993. His focus shifted to cancer biology, and he has been working in the laboratory of Robert Burk at the Albert Einstein College of Medicine studying the VHL tumor suppressor gene since 1995.

Dollfus, Hélène, M.D., Ph.D. Department of Medical Genetics, Hôpitaux Universitaires de Strasbourg, France. Dr. Dollfus is Maître de Conférence-Praticien Hospitalier in the Department of Medical Genetics and the Department of Ophthalmology in the Faculté de Médecine de Strasbourg and the Strasbourg University Hospital since 1997. Clinical and basic research topics: inherited and developmental eye diseases, genetics of deafness. She has been involved with the GEFVHL since 1995, with a special interest in the ocular manifestations of the disease for which a National study is conducted.

Erickson, Lois P., Meeting Co-Chair, Minnesota Chapter Chair, VHL Family Alliance, Bloomington, Minnesota. Lois is married and has two children: Carmen and Chad, and two grandchildren. She has VHL and so does her son Chad. Lois has served as Minnesota chair since 1993, and on the Board of Directors for five years. She chaired the first VHL meeting in Kansas City in 1994. She works at Knudson Mortgage Corporation in the Master Servicing Department. She has many hobbies and thinks of herself as a healthy person. "I don't want anyone to be as uninformed as our family was for 40 years. I will do my best to talk about VHL to everyone who will listen. I am thankful for each day that I feel well and can continue going 100 miles an hour!"

Giraud, Sophie, M.D., Ph.D. Geneticist, Hôpital Edouard Herriot, Paris, France. After completing her M.D. and Ph.D. degrees she has worked as a staff physician at the hospital in Lyon, where she works under Professor Gilbert Lenoir on various hereditary cancer syndromes. She has developed the VHL genetic testing laboratory where she works in close collaboration with Dr. Stéphane Richard. For the last two years she has been counseling people with VHL. They are developing a VHL clinic at Lyon. She helped to create the French VHL Study Group (GEFVHL), of which she is secretary.

Gläsker, Sven, Medical student, University of Freiburg, Germany. Mr. Gläsker is a medical student at the University of Freiburg and working on Dr. Hartmut Neumann's research team. He has established the largest registry of hemangioblastoma patients to date and analysed clinical and genetical data of these patients. He has worked in the Department of Neurosurgery at Stanford University Medical Center and will join the Neurosurgery team of Dr. Oldfield at the NIH for a Clinical Elective this year.

Glenn, Gladys M., M.D., Ph.D. Medical Officer, Genetic Epidemiology Branch, Cancer Diagnostic Branch, National Cancer Institute, Bethesda, Maryland. Dr. Glenn has been a clinical investigator at the National Cancer Institute since 1984. She holds concurrent appointments in Urologic Oncology, Genetic Epidemiology, and Genetics. She received a B.A. in Chemistry from Cheyney University in Pennsylvania, her M.D. and Ph.D. from the University of Pennsylvania. She completed residency and post-graduate training at Thomas Jefferson University Hospital in Philadelphia, and Johns Hopkins Oncology Center in Baltimore. At the National Cancer Institute her early molecular biology research included isolation and localization of single copy human DNA probes and their use in genetic linkage analyses and in cloning tumor suppressor genes, specifically cloning the VHL tumor suppressor gene. Since the beginning of the NCI Clinical Research Program for VHL and other forms of familial kidney cancer in 1988, Dr. Glenn has served as the primary physician for clinical evaluations,

diagnoses, treatment recommendations, referrals and genetic analyses and counseling of more than 700 at-risk and affected family members. She and her colleagues have identified a number of cancer predisposing genes and reported their clinical spectrum of disease. She is beginning to investigate factors influencing variations in growth and development of tumors in patients born with germline mutations in their VHL gene. It is hoped that this knowledge will be useful in designing specific treatments for VHL, and for prevention recommendations.

Gnarra, James, Ph.D. Associate Professor, Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans, Louisiana. Dr. Gnarra is Associate Professor of Biochemistry and Molecular Biology, and of Urology and Biometry and Genetics. He has served on the Editorial Board for the Journal of Immunotherapy and also was a Finance Committee member for the American Society of Cell Biology. His research interests include molecular genetics of cancer, renal cell carcinoma, von Hippel-Lindau disease, angiogenesis and vasculogenesis, renal development, placentation and regulation of gene expression by oxygen. He has published 62 articles and abstracts.

Goldfarb, David, M.D. Urologic Oncology, Cleveland Clinics Foundation, Cleveland, Ohio. Dr. Goldfarb is a kidney transplant surgeon with considerable experience with patients with VHL. With Dr. Andrew C. Novick, he has done extensive research on surgical techniques and outcomes for VHL in the kidney, and for transplants in people with VHL.

Gorin, Michael B., M.D., Ph.D. Associate Professor, Ophthalmology and Human Genetics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania. Dr. Gorin is the Interim Chair of the Department of Human Genetics. He graduated from Pomona College and earned his M.D. and Ph.D. degrees from the University of Pennsylvania. His primary areas of research, both clinical and basic, are in the field of ophthalmic molecular genetics, with an emphasis on hereditary retinal disorders. His major clinical research is on the molecular genetics of age-related maculopathy (ARM), and various forms of hereditary macular dystrophies and genes that have been implicated in retinal degeneration, including VHL. Dr. Gorin worked extensively with the NIH group that discovered the VHL gene during his 3.5 years at the National Eye Institute. During that time he saw over 200 VHL family members and treated numerous VHL tumors. He continues to evaluate, screen and treat VHL patients as part of his ophthalmic genetics/medical retina practice at the University of Pittsburgh.

Graff, Joyce Wilcox, Founder, Editor, VHL Family Alliance. Ms. Graff is one of the original founders of the VHL Family Alliance. She served six years as Chairman and Co-Chairman of the Board of Directors, and was named Founder Emeritus in 1999. She continues as Editor and Chairman of the Clinical Care Committee. With her late husband and her son Damon she has lived and worked with VHL since 1962. She edits the Handbook, the Newsletter, and the VHL website. An expert in electronic messaging, she is currently Vice President and Research Director in the E-Business Infrastructure research area at the Gartner Group, the world's leading high-technology advisory firm, based in Stamford, Connecticut.

Green, Jane S., Ph.D. Associate Professor of Medical Genetics, Faculty of Medicine, Memorial University, St. John's, Newfoundland. Dr. Green received her MSc degree in Genetics from the University of British Columbia in 1966, then moved to Newfoundland. She has worked at Memorial University, Faculty of Medicine since 1979, in the Ocular Genetics program, and since 1982 in Cancer Genetics as well. Her first experience with von Hippel-Lindau disease was in February 1982, when members of a large VHL family were referred to the Ocular Genetics Clinic. The VHL screening program developed because of the needs of this family, and several other VHL families identified subsequently. Dr. Green's Ph.D. thesis, completed in 1995, was entitled "Development, Implementation, and Evaluation of Clinical and Genetic Screening Programs for Hereditary Tumor Syndromes" and included VHL, the Multiple Endocrine Neoplasias, and the Hereditary Colon Cancers. The Newfoundland VHL Screening Program clearly demonstrates the value of early diagnosis and treatment of VHL tumors, and the interest of family members at risk in participating in Genetic Testing. Dr. Green continues clinical research on the hereditary Cancer Syndromes; as well as Clinical Genetic services to members of these families.

Hall, Walter A., M.D. Professor of Neurosurgery, Radiation Oncology, and Radiology, University of Minnesota, Minneapolis, Minnesota. Dr. Hall received his B.A. degree from Columbia College of Columbia University in 1979 and his M.D. degree from the College of Physicians and Surgeons of Columbia University in 1983. His neurosurgical residency was completed at the University of Pittsburgh in 1990. During his training, he spent two years at the National Institutes of Health in the Surgical Neurology Branch as a Medical Staff Fellow from 1987 to 1989. In June 1998, Dr. Hall received his M.B.A. degree from the Carlson School of Management of the University of Minnesota. He is currently in charge of the Neuro-oncology Program at the University of Minnesota and has extensive clinical and laboratory experience in developing new therapeutic

modalities for treating brain tumors. Since his arrival in Minnesota seven years ago, he has helped establish programs in stereotactic radiosurgery, fractionated stereotactic radiotherapy, blood-brain barrier disruption chemotherapy, and interventional magnetic resonance imaging.

Hammel, Pascal, M.D., Ph.D. Gastroenterology, Hôpital Beaujon, Clichy, France.. Dr. Hammel is a member of the French VHL Study Group (GEFVHL), focusing on pancreatic disease in VHL.

Harris, Adrian L., MBChB FRCP BSc MA PhD Professor of Clinical Oncology, Directory of Imperial Cancer Research Fund, Medical Oncology Unit, Churchill Hospital, Oxford, England. Dr. Harris earned his medical degree from Liverpool University Medical School, and his Ph.D. from the University of Oxford, U.K., with a dissertation "The Clinical Pharmacology of Cytosine Arabinoside." He has earned a number of prizes in biochemistry, pharmacology, and pathology, and most recently the St. Luke's Medal of the Royal Academy of Medicine in Ireland. At his laboratory in Oxford he carries out research on tumour angiogenesis and hypoxia and the development of new drug treatments for common cancers to inhibit angiogenesis. The clinical programme on cancer is being extended to try to develop antiangiogenic therapy for VHL.

Hes, Frederick, M.D., Ph.D. Dept of Genetics, Lukas Hospital in Apeldoorn, the Netherlands. Dr. Hes studied medicine at Utrecht University, where he graduated in 1995. During his studies he worked as a technician at the Laboratory of Blood Vessel Function in the University Medical Centre Utrecht, and as an information assistant in Eli Lilly, the pharmaceutical company. He also undertook practical work in Jaipur, Harare, and Aberdeen. Whilst fulfilling his compulsory national service in 1994 in the cavalry at the Bernhard Military Barracks, Amersfoot, he started the work that led to his doctoral thesis, recently published as a book, Von Hippel-Lindau disease: Clinical and Genetic Investigations in the Netherlands (Utrecht, 2000). Between 1996 and 1999 he also worked as a forensic doctor for the municipal health authority. He is now doing a residency at the Department of Internal Medicine, Apeldoorn Hospital Centre.

Heselton, Kelly Tobin, Treasurer, VHL Family Alliance, Lakeville, Minnesota. Kelly joined the Board in 1997 as Treasurer after assisting her mother, Audrey Tobin, for two years. Kelly's Mom has siblings with VHL, has known about VHL most of her life, and has fortunately led a healthy life. Kelly first heard about the VHLFA when her aunt Lois Erickson (also another Board member) contacted Joyce Graff in 1993. Kelly works at Norwest Corporation in Minneapolis, Minnesota, as a Project Manager. She has worked at Norwest for 8 years. Kelly lives in Lakeville, a suburb of Minneapolis, with her husband Dan and daughter Leah.

Ivanov, Sergey V., Ph.D. Scientist, Division of Science Applications International Corporation (SAIC), Frederick Cancer Research Center, National Cancer Institute, Frederick, Maryland. For the last four years Dr. Ivanov has been working in the Laboratory of Immunobiology with Dr. Berton Zbar and Dr. Michael Lerman. Dr. Ivanov has been involved in investigating genes responsible for kidney and lung cancers. Using an improved technology of RNA Differential Display he discovered and characterized several new genes downregulated by pVHL. For study of their products, transmembrane carbonic anhydrases IX, XII and bHLH-protein DEC1, Dr. Ivanov has been designated as Winner of the 1998 Publication Prize by Science Applications International Corporation. Dr. Ivanov is the author of more than 20 major publications.

Kaelin, William G., Jr., M.D., Ph.D. Associate Professor of Medicine, Harvard University, Dana Farber Cancer Research Institute, Boston, Massachusetts. Previously Dr. Kaelin also served as Assistant Chief of Service, Department of Medicine, Johns Hopkins Hospital, as a Clinical Associate, Dana-Farber Cancer Institute, and as Associate Physician, Brigham and Women's Hospital. Most recently Dr. Kaelin has participated as a member of the USAMRDC Breast Cancer program MB-3 Review Panel, the National Cancer Institute Breast Cancer Progress Review Group (PRG) Roundtable, Bethesda, MD, and a member of the United States Army Medical Research and Materiel Command Molecular Biology 1 Review Panel. Dr. Kaelin's major research interests are Tumor Suppressor Genes, Molecular Biology of Cancer, and Cell Cycle Regulation. Dr. Kaelin has published 36 articles in publications such as Cancer Research, Molecular Cell Biology, and Genes Development to name just a few. Dr. Kaelin is currently researching the Functional Analysis of the Von Hippel-Lindau Protein under research funding from the NIH.

Kanno, Hiroshi, M.D. Assistant Professor of Neurosurgery, Yokohama City University. Dr. Kanno is Director of Neurosurgery, Yokohama City University Hospital and an Assistant Professor of Neurosurgery, Yokohama City University School of Medicine. He has been involved in VHL research since 1993, especially in the molecular genetic mechanism of hemangioblastoma. He has focused recently on the role of VHL gene in

tumorigenesis in glioma and differentiation of neural stem cells. He has published articles about the VHL gene in Cancer Research. With Dr. Shuin and Dr. Yao he is doing epidemiological studies of VHL families in Japan.

Killary, Ann McNeill, Ph.D. Associate Professor, M.D. Anderson Cancer Research Center, Houston, Texas. Dr. Killary graduated from the University of Texas in Austin, and earned her Ph.D. from UT Graduate School of Biomedical Science, Houston, with a post-doctoral fellowship at University of Southern California Medical School, Los Angeles, and has been with the M. D. Anderson Cancer Center since 1993. She has been involved in the attempt to clone additional genes involved in the progression of sporadic and VHL associated renal cell carcinoma for many years. She is the President of the Texas Genetics Society.

Kreusel, Klaus-M., M.D. Ophthalmology, Berlin, Germany. After completing his medical degree at the Free University of Berlin, Dr. Kreusel worked as a post-doc in the Institute of clinical physiology of the Free University of Berlin. In 1993 he joined the eye clinic of Prof. Foerster. He completed his ophthalmology residency in 1998 and is currently completing his training in vitreoretinal surgery and therapy of ocular tumors.

Krzystolik, Karol A., M.D., Ph.D. Department of Genetics, Pomeranian Academy of Medicine, Szczecin, Poland. A native of Szczecin, Poland, Dr. Krzystolik is from a family of doctors. Both his parents, his older sister and younger brother are medical doctors. Beginning his medical education at the Pomeranian Academy of Medicine in Szczecin, he won a scholarship for a year of study at Trinity University in Dublin. After his graduation from the Pomeranian Medical Academy (1993) and one year of general residency, he worked in pediatric cardiology and later in pathology and genetics, where he began working on VHL. It soon emerged that VHL was considerably underdiagnosed in Poland. He began a Polish VHL Registry to coordinate diagnosis and management of VHL patients, and he now concentrates on clinical work with VHL patients. Since 1997 he is a member of the Ophthalmology Department and coordinator of the Polish VHL registry. His wife Alexandra is a biologist. They have two children, son Jaszko (4) and daughter Kinga (1)

Lee, Stephen, Ph.D. Assistant Professor of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Canada. Dr. Lee has worked on VHL since 1994, when he joined Dr. Richard D. Klausner's laboratory at the National Cancer Institute as a post-doctoral fellow. The main project of his laboratory in Ottawa consists of understanding why the VHL protein shuttles between the nucleus and the cytoplasm and why this is important for VHL tumor suppression activity. He is in the process of demonstrating that cancer causing mutations found in VHL patients, as well as patients afflicted with sporadic kidney cancer, disrupt the ability of VHL to shuttle, and function, leading to tumor development. He has published articles in the Proceedings of the National Academy of Sciences and Molecular and Cell Biology.

Lenders, J.W.M., M.D. Internal Medicine, St. Radboud University Hospital, Nymegen, Netherlands. Dr. Lenders is an internist and is currently working as a staff member in the Department of General Internal Medicine, University Medical Center St. Radboud, Nijmegen, The Netherlands. From 1991-1993 he worked as a Clinical Associate at the Clinical Neuroscience Branch (NINDS) at the National Institutes of Health, Bethesda, USA. Together with Dr. Graeme Eisenhofer they developed a plasma assay for metanephrines. Several papers were published in recent years on the metabolism of catecholamines and metanephrines in relation to pheochromocytoma. In close collaboration with members of the Urologic-Oncology Branch at the NCI a large study was carried out to assess the diagnostic value of plasma metanephrines for the diagnosis of pheochromocytoma in VHL patients.

Linehan, W. Marston, M.D. Chief of Urologic Oncology, National Cancer Institute, Bethesda, Maryland. Dr. Linehan is author or co-author on over 170 scientific publications published or in press, most dealing with kidney cancer. His primary work is the study of the genetic basis of familial and sporadic kidney cancer. This work, which began in 1986 with Dr. Berton Zbar, led to the discovery of the VHL gene. Dr. Linehan is currently studying the VHL gene to determine why inactivation of this gene leads to von Hippel-Lindau disease and how this knowledge will lead to better forms of diagnosis, prevention and therapy of this disease. Dr. Linehan is initiating clinical trials to evaluate new forms of preventative therapies in patients with von Hippel-Lindau disease.

Lisztwan, Joanna, Ph.D. Research Scientist, Novartis, Basel, Switzerland. Ms. Lisztwan graduated with honors in Biochemistry from the University of Alberta, Canada. She is currently a third year graduate student at the Friedrich-Miescher Institut, working with Dr. Wilhelm Krek. The focus of project is the VHL protein function within the cell.

Maher, Eamonn R., M.D., F.R.C.P. Professor of Medical Genetics and Head Medical and Molecular Genetics, University of Birmingham, England. Dr. Maher was previously at Cambridge University. A graduate of the University of Manchester, Dr. Maher has a special interest in the clinical and molecular aspects of familial cancer syndromes, particularly von Hippel-Lindau disease. Dr. Maher and his colleagues provide a national service for advice on the clinical management and screening, and molecular genetic testing for VHL disease. He has participated in more than 100 scientific papers and is Editor-in-Chief of the Journal of Medical Genetics. Dr. Maher and his research group are investigating the molecular genetics and function of the VHL tumour suppressor gene.

Marshall, Peggy J., Chairman, VHL Family Alliance International, Corinth, Mississippi. Peggy runs a small child-care business in her home. After attending a support meeting in June 1993 and meeting with Joyce Graff, Peggy knew that she wanted to be a part of the Alliance. Peggy started the Mississippi chapter in September 1993. She has 35 years experience with VHL, having eye and brain tumors. She has two daughters, one of whom has VHL. She has two sisters and one brother who are affected, and one unaffected sister. She also has two nephews who are affected. Over the years this extended family has depended on each other for support and strength. "We know the positive uplift of that support." In 1990, through a local doctor in her small town, she was given the name of another woman with VHL who wanted to meet her. It was remarkable to meet someone else with similar problems, and the experience taught her how valuable support groups can be. "You no longer have to feel alone with a syndrome that only your family has experienced. Through the VHL Family Alliance we can communicate and support each other." Peggy and her husband Don have both become actively involved in the VHL Family Alliance.

Maxwell, Patrick, Ph.D. University Lecturer & Consultant Physician, Centre for Human Genetics, University of Oxford, England. Dr. Maxwell completed his Ph.D. at Oxford, UK. He is a University Lecturer at the University of Oxford and Honorary Consultant Nephrologist, with a nephrology practice at the Oxford Renal Unit. With Peter Ratcliffe and Chris Pugh he runs a research group which has been investigating cellular mechanisms of oxygen-sensing for the last 10 years. Recently they have shown that the VHL gene product is essential for aspects of cellular oxygen-sensing. He was recently awarded the Mary Evelyn Lucking Prize in medicine (a national prize), and a research fellowship at Corpus Christi College, Oxford University.

Michels, Virginia, M.D. Medical Genetics, Mayo Clinic, Rochester, Minnesota. A graduate of Marquette University, magna cum laude, she received her medical degree from the Medical College of Wisconsin in Milwaukee and completed postgraduate fellowships in Milwaukee and at Baylor College of Medicine. She served on the Technical Advisory Committee for Newborn Metabolic Screening of the Minnesota State Health Department for ten years, on the medical advisory board of the National Tuberos Sclerosis Association 1987-1992, and on the Medical Advisory Board of the VHL Family Alliance since 1993. She chaired the Department of Medical Genetics from 1989 to 1999. Dr. Michels has lectured widely on her area of primary research, inherited cardiovascular disease, including visiting professorships in Okinawa, Japan, and Prague, Czechoslovakia.

Middelton, Lindsay, R.N., BSN, C.G.C. Genetic Counselor, Clinical Research Nurse, Urologic Oncology Branch, National Institutes of Health, Bethesda, Maryland. Ms. Middelton has been a genetics instructor in obstetrics, gynecology, and pediatrics. She has been with the National Human Genome Research Institute since 1994. With the Urologic Oncology Branch of the National Cancer Institute she provides genetic counseling to family members with or at risk for a Heritable Urologic Malignant Disorder. She provides verbal and written communication of genetic test results within a counseling context.

Murata, Hidetoshi, M.D. Professor of Neurosurgery, Yokohama City University. Received his medical degree cum laude from Kanazawa University School of Medicine in 1995, Board certified in Neurosurgery. With Professor Hiroshi Kanno he has written two papers on the "Relationship between the differentiation of neuronal stem cell and VHL expression" and the "Role of VHL gene in neuronal differentiation of human neuroblastoma".

Murgia, Alessandra, Ph.D. Director, Laboratory of Molecular Genetics, University of Padua, Italy. Dr. Murgia and Dr. Opocher have developed the VHL Padova network in the Azienda Ospedale Università di Padova to achieve the integrated multidisciplinary approach necessary for taking care of clinical and molecular diagnosis of VHL disease and the treatment of affected patients. Dr. Murgia earned her medical degree from the University of Padova School of Medicine, specializing in endocrinology. She earned her Ph.D. "Dottorato di Ricerca in Scienze dello Sviluppo" at the Department of Pediatrics, University of Padova. She did a post-doctoral fellowship in Genetics at the University of Pennsylvania School of Medicine, Philadelphia, and a

second at the Sestri Levante, Genova, Italy. She received the 5th Nycomed Prize for basic research from the International Society of Paediatric Oncology

Nakamura, Eijiro, M.D., Ph.D. Urologist, Faculty of Medicine, Kyoto University, Japan. Dr. Nakamura earned his MD degree at Kyoto University, Kyoto, Japan, in 1989, and finished his residency in the Department of Urology at Shizuoka City Hospital in 1993. He completed his Ph.D. in 1997 at the Department of Immunology and Cell Biology, Faculty of Medicine, Kyoto University involved in oncology and Immunology. Since 1999 he has been an Assistant Professor of the Department of Urology, Kyoto University Graduate School of Medicine, as a specialist in Oncology and Genetic Epidemiology of sporadic RCC. His publication appeared in *Genes & Development*, the *Journal of Biochemistry*, the *Journal of Immunology* and *Cancer Research*. His interest lies in finding out genetic variants that are associated with the incidence and progression of RCC.

Neumann, Hartmut P. H., M.D. Department of Nephrology, University Hospital, Alfred-Ludwigs University, Freiburg, Germany. Dr. Neumann completed his medical degree and thesis at the University of Bonn and the University of Heidelberg, with specializations in General Medicine, Pathology, and Internal Medicine, and subspecialties in Nephrology and Endocrinology. Until 1983 he worked at the Institute of Pathology, City Hospital, Ludwigshafen (Rheinland). Since 1983 he has been at the Albert-Ludwigs University in Freiburg (Breisgau), Department of Medicine, Division of Nephrology and Hypertension. His primary research project there is on inherited diseases affecting the kidney, and inheritance of hypertension. Since 1989 he has built up one of the largest studies on von Hippel-Lindau syndrome in the world, consisting of 120 patients with VHL. His publications have appeared in the *New England Journal of Medicine*, the *Lancet*, *Gastroenterology*, the journals of *Neurology*, *Neurosurgery*, and *Psychiatry*, and others. He has presented papers at the Congress of the American Society of Nephrology, the International Congress of Nephrology, the International Congress of Human Genetics, and the Congresses of the German Societies of Endocrinology and of Nephrology. He has lectured on VHL at hospitals through the U.S. and Europe including every VHLFA meeting. He organized and co-chaired the First International Symposium on VHL in Freiburg in May 1994. His tireless efforts have helped to bring a new level of attention to VHL throughout the world. Dr. Neumann was recently awarded the Franz Volhard prize for outstanding contribution to the field of Nephrology by the Society of Nephrology (*Gesellschaft für Nephrologie*) of the German-speaking countries, for his work on von Hippel-Lindau disease and inherited hypertension. In his speech to the Society, he mentioned the work of “der großartigen VHL Family Alliance, einer sehr aktiven Familieninitiative” (the magnificent VHL Family Alliance, a very active family initiative) in locating and informing families with VHL.

Nguyen, Tung, M.D. Staff Physician, Surgical Branch, National Institute of Neurological Disorders and Stroke, Bethesda, Maryland. Born in Saigon, Vietnam, Dr. Nguyen graduated from the University of Pennsylvania and earned an M.D. with distinction in research from Mt. Sinai School of Medicine in New York. He did his residency in neurosurgery and the Oregon Health Sciences University in Portland, and has been a staff physician with the National Institute of Neurological Disorders and Stroke since 1997. His work on quantification of VEGF in hemangioblastomas of the central nervous system was published with Dr. Edward Oldfield.

Niemelä, Mika, M.D., Ph.D. Attending Neurosurgeon, Helsinki University Central Hospital, Finland. Dr. Niemelä earned his medical degree from Helsinki University in 1989, and finished his residency at Helsinki Neurosurgery in 1997. He has recently finished his Ph.D. thesis entitled 'Hemangioblastomas of the CNS and retina: impact of von Hippel-Lindau disease'. He has articles published in the *Journal of Neurosurgery* and *Acta Neurochirurgica*.

Oosterwijk, Egbert, Ph.D. Associate Professor, Department of Urology, University Medical Center St Radboud, Nijmegen, The Netherlands. After his post-doctoral fellowships at Memorial Sloan-Kettering Cancer Center and the Ludwig Institute, Dr Oosterwijk joined the staff of the Urology Department in 1991 where he is responsible for the immuno- and gene-therapy program of the Department. His main research interest is in tumor immunology, particularly for renal cell carcinoma. The main focus lies in the treatment of renal cell cancers through antibody-mediated therapy.

Opocher, Giuseppe, M.D. Department of Genetics, University of Padua, Italy. Dr. Opocher and Dr. Murgia have developed the VHL Padua network in the Azienda Ospedale Università di Padova to achieve the integrated multidisciplinary approach necessary for taking care of clinical and molecular diagnosis of VHL disease and the treatment of affected patients. He has also assembled Adrennet, a discussion area for people interested in adrenal gland pathology, sponsored by the Italian society of Endocrinology.

Platt, Jay, Gunnery Sergeant, United States Marine Corps, retired, Cartersville, Georgia. Jay Platt is a retired Marine Gunnery Sergeant. He was medically retired from the Marine Corp because of his VHL condition. In August 1998, Jay began his hike of 2,160 miles of the Appalachian Trail to raise funds for fighting VHL and Cancer. Jay completed his hike on January 23, 1999 raising over \$100,000 for the VHL Family Alliance. He is the President of It's All About Attitude, Inc. and author of the book *A Time to Walk*.

Pollock, Bruce E., M.D. Neurosurgery, Mayo Clinic, Rochester, Minnesota. After completing his undergraduate degree cum laude at Allegheny College, Dr. Pollock earned his medical degree cum laude from the University of Pittsburgh School of Medicine. Also at Pittsburgh he did a residency in Neurosurgery and a Fellowship in Stereotactic and Functional Neurosurgery, and joined the faculty. He moved to the Mayo Clinic in 1997 as Director of the Mayo Clinic Gamma Knife, and served on a task force to evaluate head and neck use of stereotactic radiosurgery.

Raffel, Corey, M.D. Pediatric Neurosurgery, Mayo Clinic, Rochester, Minnesota. Dr. Raffel received his MD degree at the University of California, San Diego in 1980. He did his residency in neurosurgery at the University of California, San Francisco. He did fellowships in pediatric neurosurgery at the Hospital for Sick Children in Toronto and the University California, San Francisco from July 1986 through June of 1987. He practiced in pediatric neurosurgery at the University of Southern California School of Medicine until July of 1995 at which time he joined Mayo Clinic Department of Neurosurgery. He is now Associate Professor in Neurological Surgery at the Mayo Clinic/Mayo Foundation having been here since July of 1995. One of his main interests lies in the treatment of pediatric brain tumors through gene therapy.

Rasmussen, Patricia, B.S.W., R.N. Social Worker, Minnesota. B.S.W., L.P.N. Mrs. Rasmussen is wife, mother and grandmother of individuals who have VHL. All four of her children have VHL and one of her two grandsons have been diagnosed with VHL through DNA testing. Her husband and all of her children were diagnosed only after symptoms occurred and surgeries were required. Shortly after the discovery that her husband had VHL, it was revealed that VHL was the reason for his father dying at age 39. Feeling the urgency to gain a medical perspective on this genetic mutation, she pursued nursing at a technical college. In a continuing search to understand the social and psychological problems associated with VHL, she furthered her education at Moorhead State University by earning a degree in Social Work with a minor in Psychology. In the middle of her college education, her husband underwent surgery twice, to have cancerous tumors removed from his kidneys. Then three years later her daughters had brain tumors removed two days apart from each other. Six years after the girls had surgery her youngest son had a difficult brain tumor removed. One year later her oldest son had waited too long for a check-up and needed emergency brain surgery. This last year her youngest daughter needed another brain tumor removed. Her husband has had laser and eye surgery. Both of her sons have gone through laser on their eyes. She has worked at hospitals, clinics, and nursing homes. Currently, she is helping friends and family members to find the information and care they need to live life to the fullest.

Richard, Stéphane, M.D., Ph.D. Professor and Chairman of the Laboratory of Neuro-Oncology, Ecole Pratique des Hautes Etudes, Paris, France. Dr. Richard earned his M.D. from the Medical School of Angers and completed postgraduate training in Paris, with a Ph.D. in Histology. He was a French national level discus thrower. He holds board certification in Pathology and obtained degrees in Endocrinology, Reproductive Biology, and Genetics. His primary research interests are morphology and genetics of brain tumors. Since 1990 he and Dr. François Resche, Professor and Chairman of Neurosurgery in Nantes, created a French National Registry of VHL patients, consisting of 480 patients with von Hippel-Lindau disease. He works regularly with more than 200 patients and their families. His efforts have contributed greatly to bringing a new level of attention to VHL throughout the medical community in France. It has also enabled Dr. Richard to alert the physicians caring for people who are not aware of being at risk, so that they can take appropriate precautions. He is a founding member of VHLFA affiliate VHL France, and serves on its Medical Advisory Board. He organized and chaired the 1998 VHL Symposium in Paris. He has participated in almost 60 scientific papers and books including "Hemangioblastomas, hemangioblastomatosis and von Hippel-Lindau disease" (*Advances and Technical Standards in Neurosurgery* (1993) 20:197), "Renal Lesions and pheochromocytoma in von Hippel-Lindau disease" (*Advances in Nephrology* (1993) 23:1), and "Pheochromocytoma as the first manifestation of von Hippel-Lindau disease" (*Surgery* (1994) 116:1076). He also published many articles in French such as "Do hemangioblastomas exist outside VHL disease?" (*Neurochirurgie* (1994) 40:145, selected by the 1995 issue of the Year Book of Neuroradiology). In December 1994 Dr. Richard was awarded the Yvonne Dumonteil Prize by the French National Cancer League (Ligue Nationale contre le Cancer) for his "outstanding contribution to the clinical and epidemiologic genetics of von Hippel-Lindau disease."

Robertson, Dennis, M.D. Ophthalmology, Mayo Clinic, Rochester, Minnesota. Dr. Robertson earned his B.S., and M.D. degrees from the University of Minnesota. He served in the United States Physicians Health Service and did a residency in Ophthalmology at the Mayo Clinic. Following a fellowship in retinal diseases and surgery at Bascom Palmer Eye Institute in Miami 1968-69, he joined the staff of the Mayo Medical School where he has been a full Professor since 1980.

Rodenberg, Thomas D., Esq. Attorney at Law, Jeter, Rains & Byrn, Blue Springs, Missouri. Mr. Rodenberg is an attorney in Missouri who practices law in the area of civil rights. He has also advised numerous people on insurance issues, particularly as they relate to genetic conditions. Mr. Rodenberg has also been involved in litigation with insurance companies on the payment of benefits to those with VHL. His professional presentations include, 'Employers and the Law: What You Don't Know Will Hurt You', 1994, 'Insurance and VHL: What you don't Know can Hurt you' 1994, 'The Human Genome Project: Changing More than the Face of Genetics' 1994, and 'The Human Genome project: Kansas City, Missouri', 1999

Rothberg, Paul G., Ph.D. Director of Genetics, Children's Mercy Hospital, Kansas City, Missouri. Dr. Rothberg earned his Ph.D. from the State University of New York at Stony Brook. He did postdoctoral work at the Fox Chase Cancer Center in Philadelphia. He is currently director of the Molecular Genetics Laboratory at Children's Mercy Hospital and Professor of Medicine at the University of Missouri Kansas City School of Medicine.

Sarkar, Atom, M.D., Ph.D. Department of Neurosurgery, Mayo Clinic, Rochester, Minnesota. After completing his undergraduate degree at Brown University Dr. Sarkar went on to earn M.D. and Ph.D. degrees at the University of Miami School of Medicine. He did a neurosurgery residency at the Mayo Clinic and continues on staff at the Mayo Clinic.

Schmidt, Laura, Ph.D. Senior Scientist, Frederick Cancer Research Laboratory, National Cancer Institute, Maryland. Dr. Laura Schmidt received her Ph.D. degree from Vanderbilt University and obtained her postdoctoral training in the laboratory of Dr. James Park at Tufts University Medical School in Boston. In 1990 she joined the Laboratory of Immunobiology at the National Cancer Institute under the direction of Dr. Berton Zbar. Her research efforts have focused on the identification and characterization of genes involved in inherited kidney cancer. Her accomplishments include (1) contributions to the chromosome 3 genetic linkage map and identification of highly polymorphic microsatellite markers on chromosome 3p, (2) identification of VHL gene mutations in VHL patients and sporadic clear cell RCC, (3) location of the gene for hereditary papillary renal carcinoma (HPRC) type I by genetic linkage analysis to chromosome 7q31 and identification of mutations in the MET proto-oncogene in the germline of HPRC patients and a subset of sporadic PRC. Dr. Schmidt is currently involved in two research projects: (1) identification of the gene responsible for the Birt-Hogg-Dube (BHD) syndrome with associated kidney tumors, colonic polyps and pneumothorax by genetic linkage analysis in BHD families and (2) development of a VHL conditional knockout mouse model using the Cre/lox technology.

Seth, Prem, Ph.D. Senior Scientist, Human Gene Therapy Research Institute, Des Moines, Iowa. Dr. Seth received his undergraduate degree from the University of Delhi and his M.S. in Biochemistry from G. P. Pant University in Pantnagar, India. He earned his Ph.D. from University of Western Ontario (Canada). After four years of fellowships at the U.S. National Cancer Institute, he served as Research Associate Professor at the University of Buffalo (New York), and as a senior staff fellow at NIH. Following a year as Visiting Professor at Hokkaido University, Japan, he took his current position at the Human Gene Therapy Research Institute in Iowa. He has published nearly 100 peer-reviewed papers, mostly on adenovirus transfection, and holds a patent for a method of adenovirus-mediated cell transfection. He is currently focusing on studying the mechanisms of action of novel tumor suppressor genes using recombinant adenoviral vectors, with the hope of initiating clinical protocols using these methods.

Shuin, Taro, M.D. Professor of Urology, Kochi Medical School, Nan-koku Kochi, Japan. Dr. Shuin has been active in VHL research for more than six years. He founded the VHLFA affiliate in Japan, and has sponsored the creation and maintenance of the VHLFA website in Japan

Stebbins, Charles E., Ph.D. Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, Connecticut. Dr. Stebbins obtained a B.A. in physics in 1992 from Oberlin College in Ohio, and performed the work for his dissertation at the Memorial Sloan-Kettering Cancer Center in New York, receiving his Ph.D. in Biochemistry and Structural Biology from Cornell University in 1999. He is currently a Postdoctoral Fellow of the Damon Runyon-Walter Winchell Cancer Foundation, and studies in the laboratory of

Dr. Jorge Galan the structural foundations for the regulation of the cytoskeleton as well as signaling pathways in mammalian cells.

Stolle, Catherine A., Ph.D. Assistant Professor, Managing Director, Genetic Testing Laboratory, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania. Dr. Stolle received her Ph.D. in Biochemistry from UMDNJ-Robert Wood Johnson Medical School and trained in molecular biology at Yale University School of Medicine. She was a member of the faculty at UMDNJ-RWJ Medical School for 6 years before joining the Department of Genetics at the University of Pennsylvania School of Medicine. She started the Genetic Diagnostic Laboratory in 1994 and was certified in clinical molecular genetics in 1996 by the ACMG. Dr. Stolle has a long standing interest in the molecular basis of genetic disease and has published numerous papers on beta thalassemia, Ehler-Danlos syndrome (a genetic disease of collagen), craniosynostosis syndromes, and von Hippel-Lindau disease. The Genetic Diagnostic Laboratory currently offers clinical diagnostic testing for over 20 different disorders, with a special interest in craniosynostosis, limb-girdle muscular dystrophy, and von Hippel-Lindau.

Thompson, Geoffrey B., M.D. Associate Professor, Department of Surgery, Mayo Clinic, Rochester, Minnesota. Dr. Thompson has been with the Mayo Clinic since 1988. A graduate of Brandeis University, magna cum laude, he received his medical degree from the Medical College of Pennsylvania, cum laude, in Philadelphia. He completed postgraduate training in anatomic pathology at the University of Massachusetts Medical Center in Worcester and a residency in surgery at the Mayo Clinic in 1988. Dr. Thompson was a Mayo Foundation scholar at the University of Hong Kong, Queen Mary Hospital, in 1988, where he specialized in endocrine and hepatobiliary surgery. Dr. Thompson's primary interests focus on endocrine, gastrointestinal, and minimally invasive surgery.

Walther, McClellan M., M.D. Staff Physician, Urologic Oncology Branch, National Cancer Institute, Bethesda, Maryland. Dr. Walther and his team under Dr. W. Marston Linehan have assembled an impressive body of knowledge about hereditary causes of kidney cancer, and optimal treatments to conserve organ function. Dr. Walther has over 100 published articles, 10 Book Chapters, has made 30 presentations, 4 abstracts, and has been a guest speaker at 9 seminars/conferences.

Zbar, Berton, M.D. Chief of the Laboratory of Immunobiology, National Cancer Institute, Rockville, Maryland. Dr. Berton Zbar was trained in medicine at the Downstate Medical Center, State University of New York. He received postgraduate training in Internal Medicine and Oncology at the Utah School of Medicine. He came to the National Cancer Institute in 1965. After serving as head of the Cellular Immunity Section, he became Chief of the Laboratory of Immunobiology, DBS, NCI in 1988. Dr. Zbar's research interest is the genetics of human kidney cancer.

Index

Augsburger, James 29, 35	Faggin, Roberto 22	Imbert, Georges 6
Baker, Darren W. 13	Fallon, Maureen 9	Ivanov, Sergey V. 11, 38
Baudin, E. 15	Foerster, Michael H. 30, 32	Jääskeläinen, Juha 27
Bauer, A. L. 8	Frazier, Michael 48	Jansweijer, M.C.E. 23
Bennett, Robin L. 48	Friberg, P. 23	Joensuu, Heikki 27
Benoit, G. 14	Gallou, C. 13, 14	Joly, D. 14
Bernante, Paolo 22	Gardiman, Marina 22	Kaelin, William G. Jr. 5, 6, 38
Béroud, Christophe .. 13, 15, 27	Gaudric, Alain 27	Kanno, Hiroshi 7, 21, 28, 38
Bohling, Tom 27	Giraud, Sophie... 13, 14, 15, 27, 36	Keiser, H.R. 23
Bradley, John F. 13	Giudice, Giuseppe Lo 22	Killary, Ann McNeill 9, 39
Brauch, Hiltrud 10, 35	Gläsker, Sven 22, 36	Kim, J. 20
Brünfeld, J.P. 14	Glenn, Gladys 20, 36	Kobayashi, M. 10
Buettner, Helmut 30, 35	Gnarra, James 7, 9, 37	Kondou, Keichi 28
Burk, Robert D. 5	Goldfarb, David A. 26, 37	Koochekpour, Shahriar 9
Caldwell, Craig 7, 35	Gong, Changning 7	Krek, Wilhelm 6
Camargo, A.A. 21	Gorin, Michael B. 32, 37	Kreusel, Klaus-Martin... 30, 32, 39
Carollo, Carlo 22	Gorospe, M. 7	Krzystolik, Karol A. . 18, 39, 47
Casali da Rocha, J. C. 21, 35	Grabmaier, Karin 11	Landais, Paul 27
Chauveau, Dominique... 14, 35	Graff, Joyce Wilcox . 16, 17, 37	Lee, Stephen 5, 39
Chew, Emily 20, 30, 35	Green, Jane S. 25, 37, 47	Lemeta, Sebsebe 27
Choyke, Peter 12, 20, 29, 35	Gross, David 47	Lenders, J.W.M. 23, 39
Chrétien, Y. 14	Gstaiger, Matthias 6	Lévy, M. 13
Collins, Debra L. 47	Hall, Walter 37	Libertino, John A. 47
Colombeau, P. 14	Halley, D.J.J. 23	Libutti, Steven 20
Cook, G. A. 10	Hammel, Pascal 26, 38	Linehan, W. Marston... 12, 20, 39
Correas, J.M. 14	Harris, Adrian L. 33, 38	Link, C. 10
Couch, Vicki 19, 36, 48	Heinichen, Thomas 32	Lips, C.J.M. 23, 47
Czyzyk-Krzeska, Maria ... 8, 36	Hes, Frederick 23, 38	Lisztwan, Joanna 6, 39
Davidowitz, Eliot 5, 36	Heselton, Kelly 2, 38	Lo Giudice, Giuseppe 22
Dollfus, Hélène 27, 36	Hesseling-Janssen, A.L.W. ... 23	Luijt, R. B. van der 23
Duclos, J.M. 15	Higginbotham, J. 10	Ma, Wenbin 9
Ebersold, Michael J. 26	Hosokawa, M. 10	Maher, Eamonn R. 12, 40, 47
Eisenhofer, G. 23	Hough, C. D. 7	Majoer-Krakauer, D. F. 23
Erickson, Lois 2, 36	Huelsman, Karen 13	Marshall, Peggy J. 3, 40
Evans, Gareth 47	Huson, Susan 47	

Martella, Maddalena	12, 15, 22	Paveltich, Nikola P.	5	Simpson, A.J.G.	21
Massin, Pascale	27	Pearson, P. L.	23	Sims, Katherine B.	47
Matsutani, Masao	21	Penfornis, F.	15	Stebbins, Charles E.	5, 43
Maxwell, Patrick	8, 40	Perilongo, Giorgio	22	Stiet, J.B.	8
McGlynn, Julie	48	Piermarocchi, Stefano	22	Stolle, Catherine A.	12, 16, 19, 20, 44
Michels, Virginia V.	2, 17, 40	Pigny, P.	13	Summanen, Paula	27
Middelton, Lindsay	12, 20, 40	Platt, Jay	29, 42	Szczaluba, C.	8
Morin, P. J.	7	Plouin, P.F.	15	Tajima, Nobuyoshi	7
Muilenburg, Ann	47	Polli, Roberta	12, 15	Tatter, Stephen	48
Murata, Hidetoshi	7, 28, 40	Pollock, Bruce E.	28, 42	Taupin, Pierre	27
Murgia, Alessandra	12, 15, 22, 40	Prayer-Galletti, Tommaso	22	Terris, Benoît	26
Murphy, S.	25	Proye, C.	15	Tessarollo, Lino	9
Nagashima, Yoji	7	Raffel, Corey	42	Thompson, Geoffrey B.	24, 44
Nakamura, Eijiro	14, 41	Ragazzi, Roberto	22	Trepanier, Angela	47
Nash, J.	8	Rasmussen, Patricia	18, 42	Uhlmann, Wendy	47
Neumann, Hartmut P. H.	24, 32, 41, 47	Richard, Stéphane	13, 14, 15, 26, 27, 42, 47	Vahanian, N.	10
Nguyen, Tung T.	27, 41	Robertson, Dennis	30, 43	van den Ouweland, A. M. W.	23
Niemelä, Mika	27, 41	Rocha, J.C.C.	21, 35	van der Luijt, R. B.	23
Nishikawa, Ryo	21	Rodenberg, Thomas D.	18, 43, 49	Vance, Jeffery M.	48
Novick, Andrew C.	48	Rothberg, Paul G.	13, 43	Vilgrain, Valérie	26
O'Connor, Maureen	47	Rubinstein, Wendy	48	Wakabayashi, Toshihiko	21
Ogawa, Osamu	14	Rush, Peggy	47	Walther, McClellan	20, 23, 25, 44
Okuda, Kenji	7	Ruszniewski, Philippe	26	Wirbelauer, Christiane	6
Oldfield, Edward H.	20	Rutberg, Julie	47	Wong, G.	25
Olschwang, S.	13	Sarac, Miroslav	7	Wuast, Lynn M.	26
Oosterwijk, Egbert	11, 41	Sarkar, Atom	26, 43	Yamamoto, Isao	7, 21, 28
Opocher, Giuseppe	12, 15, 22, 41	Scaroni, Carla	22	Yao, Masahiro	21, 28
Orzan, Eva	22	Schmidt, Laura	9, 43	Yoshida, Jun	21
Ouweland, A. M. W. van den	23	Schnell, P.O.	8	Yoshida, Minoru	28
Pacak, K.	23	Schoenfeld, Alan R.	5	Zbar, Berton	9, 44
Pal, A.	10	Seth, Prem	10, 43	Zewald, R.A.	23
Paulding, W.R.	8	Sgambati, M.	12	Zhao, W.	10
		Shitara, Nobuyuki	21	Zucchetta, Pietro	22
		Shuin, Taro	21, 28, 43		

Appendix A: Clinical Care Centers

This list of participating Clinical Care Centers is current as of July 2000.

Canada: Memorial University of Newfoundland, St. John's, NF. Dr. Jane Green, Medical Genetics, Tel: +1 (709) 737-6807; Fax: +1 (709) 737-3374; E-mail: janeg@morgan.uccs.mun.ca

England:

University of Birmingham Hospital, Edgbaston, Birmingham. Professor Eamonn R. Maher; Med. Genetics, Tel: +44 121 627-2642; Fax: +44 121 627-2618; E-mail: E.R.Maher@bham.ac.uk

St. Mary's Hospital, Manchester. Dr. Gareth Evans, Genetics, Tel: +44 161 276-6206

Churchill Hospital, Oxford. Susan M. Huson, M.D., F.R.C.P., Tel: +44 (1865) 226024; Fax: +44 (1865) 226011; E-mail: shuson@immsvr.jr2.ox.ac.uk

France: Necker Hospital and Kremlin-Bicêtre Hospital, Paris. Stéphane Richard, M.D., Oncogenetics, Tel/Fax: +33 (1) 49 59 67 28; E-mail: stephane.richard@kb.u-psud.fr

Germany: Albert-Ludwigs University Hospital, Freiberg im Breisgau. Hartmut P. Neumann, M.D., Nephrology, Tel: +49 (761) 270-3363; Fax: +49 (761) 270-3778; E-mail: neumann@mm41.ukl.uni-freiburg.de

Israel: Hadassah University Hospital, Jerusalem. Dr. David Gross, Endocrinology, Tel: +972 (2) 677-7648; Fax: +972 (2) 643-7940

The Netherlands: University Hospital Utrecht. Dr. Cornelius J. M. Lips, Dept. of Internal Medicine, Tel: +31 30 250-9111; Fax: +31 30 252-3741; E-mail: c.j.m.lips@digd.azu.nl; Website in English: <http://humgen.med.ruu.nl> or in Dutch: <http://humgen.med.ruu.nl/kgcu/>

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