

**Fifth International Symposium on von Hippel-Lindau Disease
6-8th June 2002**

Final Program

Thursday 6th June

3:00 p.m. Opening Ceremony

3:30 p.m. Session 1: New Advancements in VHL Proteomics
Chairman: W.G. Kaelin Jr, USA

- 3:40 p.m.:** Cell motility: a novel regulatory function of VHL in *Drosophila melanogaster*
Vincent Dammai, Kim R. Lavenburg, Tien Hsu, USA (pag. 7)
- 4:00 p.m.:** Ubiquitin- and NEDD8-pathways regulate the E3 ubiquitin ligase activity of pVHL complex
Michael Ohh, William Y. Kim, Javid J. Moslehi, Vincent Chau, Margaret A. Read, and William G. Kaelin Jr, Canada and USA (pag. 7)
- 4:20 p.m.:** Kif-3A, a novel target of VHL
Moniek van Beest, Martijn Lolkema, Luc van Kruijsdijk, Hans Bluysen, Martijn Gebbink and Emile Voest, The Netherlands (pag. 8)
- 4:40 p.m.:** The von Hippel-Lindau tumor suppressor protein mediates ubiquitination of activated atypical protein kinaseC
Takaki Y, Okuda H, Hirai S, Iwai K, Baba M, Ohno S, Shuin T, Japan (pag. 8)
- 5:00 p.m.:** A first generation mouse model for von Hippel-Lindau disease
Laura Schmidt, Wenbin Ma, Lino Tessarollo, Masaya Baba, Nirmala Sharma and Berton Zbar, USA and Japan (pag. 9)
- 5:20 p.m.** Coffee Break
- 5:40 p.m.:** von Hippel-Lindau Tumor Suppressor Binds to Microtubules and Promotes Microtubule Stability
Hergovich, A., Lisztwan, J., Barry, R., Ballschmieter, P., and Krek, W, Switzerland (pag. 9)
- 6:00 p.m.:** Role of VHL in the Formation of beta 1 Integrin Fibrillar Adhesions
Miguel A. Esteban-Barragán, Pilar Ávila and Manuel O. Landázuri, Spain (pag. 10)
- 6:20 p.m.:** The TRC8 Hereditary Kidney Cancer Gene Suppresses Growth and Functions with VHL in a Common Pathway
RM Gemmill, LT Bemis, JP Lee, MA Sozen, A Baron, C Zeng, PF Erickson, JE Hooper and HA Drabkin, USA (pag. 10)
- 6:40 p.m.:** Comparative evaluation of the anti-tumor activity of angiogenesis inhibitors delivered by viral vectors
Stefano Indraccolo, Eleonora Gola, Antonio Rosato, Sonia Minuzzo, Walter Habeler, Veronica Tisato, Valeria Roni, Giovanni Esposito, Alberto Amadori, and Luigi Chieco-Bianchi, Italy (pag. 11)
- 7:00 p.m.:** Neuronal differentiation of neural stem cells by VHL gene induction and neuronal regeneration with grafting the VHL-induced cells
Hiroshi Kanno, Hidetoshi Murata, Yoshihide Tanaka, Toshiro Mimura, Isao Yamamoto, Nobuyoshi Tajima, Kyohei Murakami, Mari Dezawa, and Hitoshi Yamada, Japan (pag. 11)
- 7:20 p.m.:** Role of VHL in regulating HIF-1: implications for oxygen-sensing and VHL disease.
Patrick Maxwell, England (pag. 12)

7:40 p.m.: General Discussion

8:00 p.m. Dinner Buffet

Friday 7th June

8:30 a.m. Session 2: *Genotype/Phenotype Alignment*

Chairman: E.R.Maher, England

- 8:30 a.m.:** Frequent losses of chromosomal arm 6q in capillary hemangioblastoma
Lemeta S, Aalto Y, Niemelä M, Jääskeläinen J, Sainio M, Kere J, Knuutila S, Böhling T, Finland (pag. 12)
- 8:50 a.m.:** Association of the PGP Transporter MDR1C3435T Polymorphism With The Susceptibility To Renal Epithelial Tumors
Michael Siegsmond, Ulrich Brinkmann, Elke Schöffeler, Gregor Weirich, Matthias Schwab, Michel Eichelbaum, Peter Fritz, Oliver Burk, Jochen Decker, Tim M Jaeger, Peter Alken, Uwe Rothenpieler, Reinhold Kerb, Sven Hoffmeyer, Hiltrud Brauch, Germany (pag. 13)
- 9:10 a.m.:** VHL Alterations Associated with Good Prognosis in Sporadic Clear-cell Renal Carcinoma
Masahiro Yao, Minoru Yoshida, Takeshi Kishida, Noboru Nakaigawa, Masaya Baba, Kazuki Kobayashi, Takeshi Miura, Masatoshi Moriyama, Yoji Nagashima, Yukio Nakatani, and Kei-ichi Kondo, Japan (pag. 13)
- 9:30 a.m.:** Analysis of genotype-phenotype correlations in VHL patients with pancreatic neuroendocrine tumors.
Pascal Hammel, Sophie Giraud, Christophe Bérout, Valérie Vilgrain, Alain Sauvanet, Benoît Terris, Jean-Michel Corréas, Patricia Niccoli-Sire, Maha Kacem, Philippe Ruzsiewicz and Stéphane Richard for the French VHL Study Group, France and Tunisie (pag. 14)
- 9:50 a.m.:** Somatic point mutation of the wild type allele detected in tumors of patients with VHL germline deletion
Z. Zhuang, S. Huang, S. Pack, C. A. Koch, I. A. Lubensky, E. H. Oldfield, A. O. Vortmeyer, USA (pag. 14)
- 10:10 a.m.:** Germ-line mutations in nonsyndromic pheochromocytoma
Neumann HPH, Baush B, McWhinney SR, Bender BU, Gimm O, Franke G, Schipper J, Klisch J, Althoefer C, Zerres K, Januszewicz A, Eng C. Germany, Poland and USA (pag. 15)
- 10:30 a.m.:** Fine structure mapping of chromosome 1 in sporadic and familial pheochromocytoma
G.Opocher, F.Schiavi, A.Vettori, A.Calderan, B.Vianello, A.Murgia, M.Martella, A.Taccaliti, F.Mantero, M.L.Mostacciolo, Italy (pag. 15)

10:50 a.m.: *General Discussion*

11:30 a.m. Coffee Break

12:00 a.m. Session 3: *Genotyping*

Chairman: C.Stolle, USA

- 12:00 a.m.:** Genotype-phenotype correlations in von Hippel-Lindau disease - the NCI experience.
Berton Zbar, Cathy Stolle, Gladys Glenn, McClellan Walther, Peter Choyke, Emily Chew, Laura Schmidt and Marston Linehan, USA (pag. 16)
- 12:15 a.m.:** Detection of germline deletions in the von Hippel Lindau TUMOR suppressor gene using multiplex polymerase chain reaction of short fluorescent fragments
S. Giraud, S. Georges, S. Pinson, O.M. Sinilnikova, F. Penforis, D. Chauveau, B. Gilbert, D. Lacombe, A. Dutour, A. Calender, S. Richard and the French VHL study Group, France (pag. 16)
- 13:30 a.m.:** Contribution of sporadic cases to the prevalence of Von Hippel-Lindau (VHL) gene germline mutations in the Netherlands
F.J. Hes, A.M.W. van den Ouweland, R.A. Zewald, A.L.W. Hesseling-Janssen, M. Doleman, H.J. Eussen, D.J.J. Halley, C.J.M. Lips, J.K. Ploos van Amstel, P.L. Pearson, D.F. Majoer-Krakauer, R.B. van der Luijt, The Netherlands (pag. 17)
- 12:45 a.m.:** Mutational analysis of the VHL gene: results clinical impact and implications of a validated molecular approach
Martella M, Polli R, Leonardi E, Finco F, Schiavi F, Opocher G, Murgia, Italy (pag. 17)

1:00 p.m.: General Discussion

1:30 p.m. Lunch

Friday 7th June

2:30 p.m. Session 5: VHL Posters

Chairman: A.Murgia, Italy & F.J.Hes, The Netherlands

- #1 Immunohistochemical findings of the nitric oxide synthase and p53 protein expression in renal cell carcinomas with VHL gene mutations
Mutsuo Furihata, Shingo Ashida, Yuji Ohtsuki, Japan (pag. 18)
- #2 Molecular mechanism of contact inhibition of cell growth by tumor suppressor protein pVHL
Masaya Baba, Syu-ichi Hirai, Takeshi Kishida, Noboru Nakaigawa, Kazuki Kobayashi, Masahiro Yao, Yoshinobu Kubota, Shigeo Ohno, Japan (pag. 18)
- #3 Regulation of intercellular Junction Assembly and Paracellular Permeability by VHL
Pilar Ávila, Miguel A. Esteban-Barragán and Manuel O. Landázuri, Spain (pag. 19)
- #4 Molecular Pathogenesis of CNS Hemangioblastoma
Gläsker S, Bender BU, Apel TW, van Velthoven V, Mulligan LM, Zentner J, Neumann HPH, Canada and Germany (pag. 19)
- #5 Treatment policy for spinal hemangioblastoma with von Hippel-Lindau disease
Hiroshi Kanno, Isao Yamamoto, Masahiro Yao, Ryo Nishikawa, Masao Matsutani, Toshihiko Wakabayashi, Jun Yoshida, Nobuyuki Shitara, Taro Shuin, Japan (pag. 20)
- #6 Impact of the molecular and clinical screening in Brazilian families with von Hippel-Lindau disease (VHL)
JCC Rocha, AA Camargo, RLA Silva, AJG Simpson, Brazil (pag. 20)
- #7 Clinical features in the VHL disease in Japan: A survey for the onset of age on the VHL associated diseases.
Taro Shuin, Yasuyuki Takaki, Masahiro Yao, Hiroshi Kanno, Isao Yamamoto, Ryo Nishikawa, Masao Matsutani, Toshihiko Wakabayashi, Jun Yoshida, Nobuyuki Shitara, Japan (pag. 21)
- #8 A Register Approach to the Management of Von Hippel Lindau Disease.
Carol Giblin, Fiona Laloo, Gareth Evans, England (pag. 21)
- #9 Hippel-Lindau disease in Hungary.
Pfliegler G., Fazakas F., Balázs E., Nagy V, Berta A, Hungary (pag. 22)
- #10 Description of the von Hippel-Lindau disease in three spanish families
Karina Villar Gómez de las Heras, Spain (pag. 22)
- #11 Partial nephrectomy: alternative treatment for renal cell carcinoma in patients with Von Hippel-Lindau disease
RJA van Moorselaar, FJ Hes, R Lazarov, CJM Lips, The Netherlands (pag. 23)
- #12 Caring for Families with VHL & shy; the experiences of Guy's Hospital, London
S. Watts, F. Kavalier, S. Hodgson, England (pag. 23)
- #13 Moleculargenetic testing for VHL in vascular retinal tumors
Kreusel K.-M., N.E. Bechrakis, H.P.H. Neumann, M.H. Foerster, Germany (pag. 24)
- #14 Retinal hemangioma in the von Hippel-Lindau (VHL) disease. Identification, evaluation and management. A review
S.Piermarocchi, G. Lo Giudice, L. Caretti, E. Pilotto Italy (pag. 24)
- #15 Moleculargenetic and clinical investigation of mutations in the *SDHB* Gene
B. Bausch, S. McWhinney, B. Bohnert-Iwan, G. Franke, M. Peczkowska, J. Schipper, A. Januszewicz, C. Eng, H.P.H. Neumann, Germany, Poland and USA (pag. 25)

4:30 p.m. Coffee Break

5:00 p.m. Guided Visit to the Old Abbey

8:30 p.m. Social Dinner at the Air Museum, San Pelagio, Padova

Saturday 8th June

8:30 a.m. Session 6: Clinics 1

Chairman: S.Richard, France

- 8:30 a.m.:** 18Fluoro-DOPA whole-body positron emission tomography for detection of pheochromocytomas: initial results
Stefan Hoegerle, Egbert Nitzsche, Carsten Altehoefer, Nadir Ghanem, Tanja Manz, Ingo Brink, Martin Reincke, Ernst Moser, Hartmut P.H. Neumann, Germany (pag. 25)
- 8:45 a.m.:** The natural history of central nervous system hemangioblastomas in von Hippel-Lindau disease (VHL)
John E. Wanebo, Russell R. Lonser, Gladys M. Glenn, Edward H. Oldfield, USA (pag. 26)
- 9:00 a.m.:** New Insights into the Histogenesis of Hemangioblastoma
A. O. Vortmeyer, K. Yuan, E. H. Oldfield, Z. Zhuang, USA (pag. 26)
- 9:15 a.m.:** Germline mutation of the SDHB gene associated with pheochromocytoma of urinary bladder.
Mariola Peczkowska, Neumann HPH, Kabat M, Cybulska I, Janaszek-Sitkowska H, Prejbisz A, Januszewicz M, Ciela W, Januszewicz A, Poland and Germany (pag. 27)

9:30 a.m. Session 7: Clinics 2

Chairman: H.P.H. Neumann, Germany

- 9:30 a.m.:** VHL in Italy: what has been done and what needs to be done
Murgia A, Martella M, Schiavi F, Piermarocchi S, Lo Giudice G, Scienza R, Berlucchi S, Carollo C., Orzan E, Prayer Galletti T, Pagano F, Opocher G, The VHL Padova Network, Italy (pag. 27)
- 9:45 a.m.:** National implementation of programmatic prevention of complex hereditary tumour syndromes
C.J.M. Lips, F.J.Hes and R.B. van der Luijt, The Netherlands (pag. 28)
- 10:00 a.m.:** Searching for the von Hippel-Lindau disease (VHL): two important differential diagnoses
JCC Rocha, AA Camargo, RLA Silva, IL Cendes, AJG Simpson, Brazil (pag. 28)
- 10:15 a.m.:** Is endolymphatic sac tumor a major criterion for diagnosis of von Hippel Lindau disease ?
Kathlyn Marsot-Dupuch, Frédéric Portier, Christine Le Pajolec, Sophie George, Sophie Giraud, Serge Bobin, Philippe Capelle, Pierre Lasjaunias, Gérard Benoît, Stéphane Richard, France (pag. 29)

10:30 a.m.: General Discussion

11:00 a.m. Coffee Break

11:20 a.m.: Round Table: an update of follow-up and intervention criteria

S.Richard, HPH Neumann, F.Pagano, R.Scienza, KM Kreusel

12:50 a.m.: Free Opinion-Exchange between Patients and Doctors

1:30 p.m.: Lunch

3:00 p.m. Session 8: News on the VHL Therapy

Chairman: C.J.M. Lips, The Netherlands

- 3:00 p.m.:** Treatment with halofuginone results in marked growth inhibition of a VHL pheochromocytoma ex-vivo.
David J. Gross, Israel Reibstein, Lola Weiss, Shimon Slavin, Hagit Dafni, Michal Neeman and Arnon Nagler, Israel (pag. 29)
- 3:15 p.m.:** Surprising effects of anti-VEGF receptor therapy in von Hippel-Lindau patients
Stéphane Richard, Jean-François Girmens, Laure Croisille, Jeannine Yvart, Nicole Casadeval, Pascal Eschwège, Nozar Aghakhani, Philippe David, Guy Allègre, Paul Scigalla, Olivier Hermine, Alain Gaudric, France (pag. 30)

Saturday 8th June

3:30 p.m. Session 6: News from VHL Family Alliances
Chairman: J.Graff, USA

3:45 p.m.: Foundation and Development of the VHL Alliance in Spain

Jesusa Martínez Gómez and Karina Villar Gómez de las Heras, members of the VHL Spanish group, Spain (pag. 30)

4:00 p.m.: VHL in Germany. Dealing with Psychological and Social Aspects of VHL

Gerhard Alsmeyer, Chair, German VHL Alliance, Germany (pag. 30)

4:15 p.m.: General Discussion

4:30 p.m.: Symposium Highlights and Closing Remarks

Abstracts

Cell motility: a novel regulatory function of VHL in *Drosophila melanogaster*

Vincent Dammai^{1,2}, Kim R. Lavenburg^{1,2}, Tien Hsu^{2,3}

¹Equal contributors; ²Laboratory of Cancer Genomics, Hollings Cancer Center; ³Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, SC 29425

Our previous work on the *Drosophila* homologue of VHL provided an *in vivo* confirmation for its role in modulating vasculo/angiogenesis. However, the pleiotropic phenotypes of the VHL disease and VHL knockout mice indicate that VHL may regulate multiple cellular functions. We have noted that VHL knock-down mutation in *Drosophila* not only leads to ectopic looping of the vasculature but also results in dispersion of the vascular cells, suggesting that VHL may negatively regulate cell motility. To test this hypothesis, we conducted a yeast two-hybrid screen for *Drosophila* pVHL interacting proteins. The majority of the positive clones (i.e., verified by interaction with pVHL *in vivo*) are either components or regulators of the cytoskeleton. Here we focus on two particularly interesting ones. First, the AWD protein is the homologue (77% identity) of human metastasis inhibition factor nm23. AWD/nm23 is a nucleoside diphosphate kinase that supplies GTP to the Ras-like GTPase Rac. Rac-mediated signaling can effect rearrangement of actin filaments, a process essential for cell migration. awd loss-of-function mutation results in reduced vascular tubule network while a gain-of-function awd allele induces ectopic branching and "budding". Another pVHL-interacting protein is the non-muscle myosin light chain II, encoded by the sqh gene, which undergoes redistribution during cell migration. We show that during ovarian development VHL knock-down phenocopies the sqh mutation in which follicular epithelial migration is impaired.

Ubiquitin- and NEDD8-pathways regulate the E3 ubiquitin ligase activity of pVHL complex

Michael Ohh¹, William Y. Kim², Javid J. Moslehi², Vincent Chau³, Margaret A. Read⁴, and William G. Kaelin Jr^{2,5}

¹Department of Laboratory Medicine and Pathobiology, University of Toronto,

²Department of Adult Oncology, Dana-Farber Cancer Institute, Harvard Medical School,

³Department of Cellular and Molecular Physiology, Pennsylvania State College of Medicine,

⁴Millennium Pharmaceuticals, and ⁵Howard Hughes Medical Institute

The product of the von Hippel-Lindau (VHL) gene, pVHL, forms a multimeric complex (VEC) with elongins B and C, Cul2, and Rbx1. The VEC complex functions as an E3 ubiquitin ligase to target the α subunits of hypoxia-inducible factor (HIF) transcription factor for ubiquitylation. This process marks HIF α for subsequent destruction by the 26S proteasome. The recognition of HIF α by VEC is via the β domain of pVHL and requires the oxygen-dependent hydroxylation of HIF α at conserved Proline residues 564 and 402. Consequently, tumour cells lacking functional pVHL inappropriately overproduce the products of HIF target genes such as VEGF, which is a major angiogenic factor, and thereby likely accounts for the hypervascular nature of VHL-associated neoplasms. However, the molecular mechanisms that control the activity of the VEC complex are unknown. Here, we show that (1) an ubiquitin-like molecule NEDD8 covalently modifies Cul2 at Lysine 689 under physiologic conditions; (2) hUbc12 is the necessary E2 NEDD8-conjugating enzyme for this modification; and (3) ongoing neddylation is required for VEC function *in vitro* and for the degradation of VEC substrate HIF α *in vivo*.

Kif-3A, a novel target of VHL

Moniek van Beest, Martijn Lolkema, Luc van Kruijsdijk, Hans Bluysen, Martijn Gebbink and Emile Voest.
Department of Medical Oncology, Division of Internal Medicine, University Medical Center Utrecht, The Netherlands.

The von Hippel-Lindau tumor suppressor protein mediates ubiquitination of activated atypical protein kinase C.

Okuda H¹, Takaki Y¹, Hirai S², Iwai K³, Baba M², Ohno S², Shuin T¹.

¹Department of Urology, Kochi Medical School, Kochi, Japan. ²Department of Molecular Biology, Yokohama City University School of Medicine Yokohama, Japan. ³Department of Molecular Biology Osaka City University Medical School, Osaka Japan.

The von Hippel-Lindau tumor-suppressor protein (pVHL) forms a protein complex (VCB-Cul2) with elongin C, elongin B, Cul-2, and Rbx1, which functions as a ubiquitin-protein ligase (E3). The alpha-subunits of the hypoxia-inducible factors have been identified as targets for the VCB-Cul2 ubiquitin ligase. However, a variety of cellular defects caused by the depletion of pVHL cannot be explained solely by the ubiquitin-mediated degradation of hypoxia-inducible factor-alpha. We show here that a member of the atypical protein kinase C (PKC) group, PKClambda, is ubiquitinated by the pVHL-containing E3 enzyme. An active PKClambda mutant is ubiquitinated more extensively than wild-type PKClambda in HEK293 cells, and the ubiquitination is further enhanced by the overexpression of pVHL. The activation of wild-type PKClambda by serum stimulation of cells enhances the ubiquitination of the protein, supporting the notion that active PKClambda is preferentially ubiquitinated by VCB-Cul2 ubiquitin ligase. Furthermore, we show that PKClambda can be ubiquitinated in vitro in a cell-free ubiquitination assay using purified recombinant components including VCB-Cul2. Given the known function of aPKC in the regulation of cell polarity and cell growth, PKClambda may be a target of pVHL in its function as a tumor suppressor.

A first generation mouse model for von Hippel-Lindau disease

Laura Schmidt, Wenbin Ma, Lino Tessarollo, Masaya Baba, Nirmala Sharma and Berton Zbar. IRSP, SAIC-Frederick, Inc., Laboratory of Immunobiology, and Mouse Cancer Genetics Program, NCI Frederick, Frederick, Maryland, USA; Dept. of Urology, Yokohama City Univ. School of Medicine, Yokohama, Japan

Von Hippel-Lindau disease is an inherited multisystem neoplastic disorder characterized by a predisposition to develop vascular tumors of the retina, cerebellum, brain stem, and spinal cord. In addition to vascular tumors, clear cell carcinomas of the kidney, pheochromocytomas, and cystadenomas of the pancreas and epididymis also occur in patients affected with VHL. Homozygous deletion of the VHL gene was shown to cause embryonic lethality in mice. We have developed a mouse model for von Hippel-Lindau disease using Cre/lox site-specific recombination technology to conditionally inactivate the VHL gene. Mouse embryonic stem cells were targeted, by homologous recombination, with a VHL target vector in which exons 2 and 3 of the mouse VHL gene were flanked by lox-P sites (floxed). Mice were produced with a germline VHL floxed allele. VHL^{flox/+} and VHL^{flox/flox} mice were viable, fertile and developed normally. VHL mRNA size and expression level in the tissues of VHL^{flox/+} and VHL^{flox/flox} mice were comparable to wild-type litter mates. Several Cre transgenic mice were tested for Cre tissue expression using ROSA26 reporter mice. In order to mimic VHL disease, we selected a transgenic mouse with a beta-actin promoter-driven Cre recombinase that was expressed in a mosaic pattern in multiple organs. Breeding the Cre transgenic mice with VHL^{flox/+} mice produced VHL^{deleted/+} mice carrying the beta actin Cre transgene. We have produced 300 mice by breeding VHL^{flox/flox} mice with VHL^{deleted/+}, beta actin Cre^{+/+} mice. Nine percent of mice produced from this cross carry the VHL^{flox/deleted}, Cre^{+/+} genotype. About 1/3 of the mice with the VHL^{flox/deleted}, beta actin Cre^{+/+} genotype, who have reached 6 months of age, have died from vascular lesions in the liver. An anatomic and histologic evaluation of these mice will be presented. A mouse model for human VHL disease may prove useful for future testing of cancer treatments and gene therapies. Funded in part by DHHS#NO1-CO-12400.

von Hippel-Lindau Tumor Suppressor Binds to Microtubules and Promotes Microtubule Stability

Hergovich, A., Lisztwan, J., Barry, R., Ballschmieter, P., and Krek, W.

Friedrich Miescher Institut for Biomedical Research, Maulbeerstrasse 66, CH-4058 Basel, Switzerland.

von Hippel-Lindau (VHL) disease is a dominantly inherited cancer syndrome caused by germline mutation of the VHL tumor suppressor gene and is characterized by predisposition to multiple tumors of the eyes and central nervous system (hemangioblastomas), adrenal chromaffin cells (pheochromocytoma), kidneys (renal cell carcinoma) and other organs. Different germline VHL mutations are associated with different site-specific risks of cancer. The cell biological basis for these variations in tumor risks is poorly understood but is likely a reflection of distinct changes in pVHL function in the corresponding mutants. Using a series of specific antibodies raised against human and mouse pVHL, we found that the corresponding proteins co-localize with the microtubule network in vivo. This association is disrupted by drugs that destabilize microtubules (e. g. nocodazole) but enhanced by ones that stabilize microtubules (e. g. taxol). Consistent with these findings, pVHL associates also with microtubules in vitro. Importantly, functional assays revealed that wild-type pVHL increases microtubule stability in vivo, whereas certain naturally-occurring pVHL mutants are defective in this regard. The significance of these findings with respect to understanding the complex genotype-phenotype relationships underlying VHL disease will be presented.

Role of VHL in the Formation of beta 1 Integrin Fibrillar Adhesions

Miguel A. Esteban-Barragán, Pilar Ávila and Manuel O. Landázuri.

Servicio de Inmunología, Hospital de la Princesa, 28006 Madrid, Spain

VHL is mutated in the von Hippel-Lindau syndrome and in most sporadic renal cancers. As previously reported, VHL is required for the assembly of an extracellular fibronectin matrix, although the mechanism remains unknown. We have demonstrated that 786-O renal cancer cells are unable to organize an adequate matrix even in the presence of an excess of exogenous fibronectin. Since the formation of integrin fibrillar adhesions plays a pivotal role in the organization of extracellular fibronectin, we next examined the expression and subcellular distribution of integrins in VHL(-) cells and their wild-type VHL stably transfected counterparts. The levels of beta 1 and alpha v integrins were increased in VHL(-) cells when compared to VHL(+) transfectants. Early after plating, both groups of cells assembled classic alpha v focal contacts. As the culture advanced, alpha v integrins partly relocated to the intercellular junctions in VHL(+) transfectants, which then developed large beta 1 fibrillar-type adhesions and anchored firmly to the substrate. In contrast, VHL(-) cells were unable to assemble beta 1 fibrillar adhesions, and alpha v focal contacts remained unchanged along any stage of the culture. Exogenous activation of beta 1 integrins, partly restored the capability of VHL(-) cells to assemble fibrillar adhesions and fibronectin fibers. Finally, pulse-chase studies of metabolically labeled 786-O cells revealed that the maturation of the common beta 1 integrin chain was delayed in VHL(-) cells, when compared to VHL(+) cells. In conclusion, our results show that VHL is essential for the formation of beta 1 fibrillar adhesions, and help to explain the abnormal matrix organization and increased motility of VHL(-) renal cancer cells.

The TRC8 Hereditary Kidney Cancer Gene Suppresses Growth and Functions with VHL in a Common Pathway.

RM Gemmill¹, LT Bemis¹, JP Lee¹, MA Sozen¹, A Baron³, C Zeng³, PF Erickson¹, JE Hooper² and HA Drabkin¹.

¹Div. Medical Oncology, ²Dept. of Cellular & Structural Biology, ³Dept. of Preventive Medicine & Biometrics, University of Colorado Health Sciences Center, 4200 E. 9th Avenue, Denver, CO 80262, USA.

VHL is part of a SCF-like E3-ubiquitin ligase complex with "gatekeeper" function in renal carcinoma. However, in wt-VHL tumors, no mutations have been identified in known VHL interacting proteins. We previously reported that the TRC8 gene was interrupted by a t(3;8) translocation in a family with hereditary renal and non-medullary thyroid cancer. TRC8 encodes a multi-membrane spanning protein with partial similarity to the Hedgehog receptor, Patched, and in addition contains a ring-H2 finger with in vitro ubiquitin ligase activity. We isolated the Drosophila homolog, DTrc8, and studied its function by genetic manipulations and a yeast 2-hybrid screen. Human and Drosophila TRC8 proteins localize to the endoplasmic reticulum. Loss of either DTrc8 or DVhl by RNAi resulted in an identical ventral midline defect. Direct interaction between DTrc8 and DVhl was confirmed by GST-pulldown and co-immunoprecipitation experiments. CSN-5/JAB1 is a component of the signalosome, recently shown to regulate SCF function. In addition, JAB1 has been shown to regulate the nuclear export of p27kip1 in a proteasome dependent manner. From the 2-hybrid screen and subsequent confirmatory studies, we found that DTrc8 physically interacts with CSN-5. In VHL mutant cell lines, or wt-VHL cell lines treated with proteasome inhibitors, the perinuclear pattern of JAB1 staining was lost. Lastly, overexpression of DTrc8 in the wing or eye imaginal discs inhibited growth consistent with its presumed role as a tumor suppressor gene. Thus, VHL, TRC8, and JAB1 appear intimately linked, both physically and functionally, in the development of kidney cancer.

Comparative evaluation of the anti-tumor activity of angiogenesis inhibitors delivered by viral vectors

Stefano Indraccolo^{1,2}, Eleonora Gola², Antonio Rosato², Sonia Minuzzo², Walter Habeler², Veronica Tisato², Valeria Roni², Giovanni Esposito², Alberto Amadori², and Luigi Chieco-Bianchi².

¹ IST-Viral and Molecular Oncology Section-Padova, Padova;

² Department of Oncology and Surgical Sciences, University of Padova, Padova;

The administration of different angiogenesis inhibitors by gene transfer has been shown to result in inhibition of tumor growth in animal tumor models, but the potency of these genes has been only partially evaluated in comparative studies to date. To identify the most effective anti-angiogenic molecule for delivery by retroviral vectors we investigated the effects of angiostatin, endostatin and interferon(IFN)- α_1 gene transfer in mouse models of breast cancer induced neovascularization and tumor growth. Moloney leukemia virus-based retroviral vectors for expression of murine angiostatin, endostatin and IFN- α_1 were generated, characterized, and used to transduce human breast cancer cell lines (MCF7 and MDA-MB435). Secretion of the recombinant proteins was confirmed by biological and western blotting assays; their production did not impair in vitro growth of these breast cancer cells nor their viability, and did not interfere with the expression of angiogenic factors. However, primary endothelial cell proliferation and migration in vitro were inhibited by supernatants of the transduced cells containing angiostatin, endostatin, and IFN- α_1 . Stable gene transfer of the IFN- α_1 cDNA by retroviral vectors in both MCF7 and MDA-MB435 cells resulted in a marked and long-lasting inhibition of tumor growth in nude mice that was associated with reduced vascularization. Endostatin reduced the in vivo growth of MDA-MB435 but not MCF7 cells, despite similar levels of in vivo production and angiostatin did not impair the in vivo growth of either tumor cell line. These findings indicate heterogeneity in the therapeutic efficacy of angiostatic molecules delivered by viral vectors and suggest that gene therapy with IFN- α_1 and endostatin might be useful for treatment of breast cancer. The implications of these results for the design of novel therapeutic approaches to VHL-associated tumors will be discussed.

Neuronal differentiation of neural stem cells by VHL gene induction and neuronal regeneration with grafting the VHL-induced cells

Hiroshi Kanno, Hidetoshi Murata, Yoshihide Tanaka, Toshiro Mimura, Isao Yamamoto, , Nobuyoshi Tajima, Kyohei Murakami, Mari Dezawa, and Hitoshi Yamada

Departments of Neurosurgery, Bacteriology, Orthopedics, Anatomy, and Neurology, Yokohama City University School of Medicine, Yokohama, Japan

VHL gene induction causes neuronal differentiation in neuronal stem cells (NSCs). In contrast, a VHL mRNA antisense oligonucleotide inhibits differentiation of NSCs and up-regulates their cell cycle. Addition of GDNF to culture medium transforms most of VHL-gene induced NSCs to dopamine secreting cells showing immunoreactivity to tyrosine hydroxylase (TH). An electrophysiological study with patch clamp method reveal that the VHL-gene induced NSCs show sodium-potassium current equal to mature neurons. Grafting the VHL-gene induced NSCs into brain of Parkinson model rat markedly improves the behavioral symptom. Most of the grafted cells differentiate neurons showing immunoreactivity to both MAP-2 (mature neuronal marker) and TH. In conclusion, VHL-gene induced NSCs which are grafted into central nervous system form neuronal network and function as functional neurons. The grafting the cells would be clinically useful for intractable neuronal diseases such as Parkinson's disease, cerebral infarction, and spinal cord injury.

Role of VHL in regulating HIF-1: implications for oxygen-sensing and VHL disease.

Patrick Maxwell

Renal Section, Imperial College of Science, Technology and Medicine, Hammersmith Campus, Du Cane Road, London W12 0NN

Several lines of evidence suggest that genetic events in cancer might amplify normal cellular responses to oxygen mediated by Hypoxia-Inducible Factor-1. Clinical manifestations of VHL disease, together with effects on gene expression suggested VHL loss-of-function could act in this way. This led to the realisation that VHL is required for HIF regulation, acting as the recognition component of a ubiquitin ligase complex. This relationship is highly conserved in evolution.

The recognition that VHL is centrally involved in responses to oxygen has led to a breakthrough in understanding the oxygen-sensing mechanism itself. HIF capture is regulated by the enzymatic hydroxylation of two specific conserved prolyl residues. The enzymes are a new family of 2 oxo-glutarate dependent dioxygenases that act as cellular oxygen sensors.

What does the role of VHL in HIF regulation tell us about clinical manifestations of VHL mutations? One approach is to study effects of mutations on function. Those associated with Type 1, 2A or 2B VHL disease abrogate HIF regulation. In contrast, mutations associated with pheochromocytoma alone (type 2C) do not. Another approach has been to study the early events in the kidney of individuals with VHL mutations. We found that HIF activation is manifest in many morphologically normal single cell profiles within the renal tubules. In comparison, dysplastic lesions, cystic lesions, and tumors showed evidence of additional mechanisms of HIF activation. Detection of cells with constitutive HIF activation identified a large number of previously unrecognised foci of VHL inactivation. In proximal tubules these were almost entirely unicellular, whereas multicellular foci were almost exclusively in the distal nephron. This establishes that HIF activation has the potential to play a role in both early and later stages of VHL associated tumorigenesis. Furthermore, effects of VHL inactivation on proliferation appear site specific within the nephron.

Frequent losses of chromosomal arm 6q in capillary hemangioblastoma

Lemeta S, Aalto Y, Niemelä M, Jääskeläinen J, Sainio M, Kere J, Knuutila S, Böhling T. Departments of Pathology, Medical Genetics and Neurosurgery, University of Helsinki, Helsinki, Finland

Capillary hemangioblastomas (CHBs) of the central nervous system, the most common tumor in von Hippel-Lindau (VHL) disease, usually show mutations in the VHL tumor suppressor gene on chromosome 3p25-26. Studies on other chromosomal changes occurring in CHBs are few. In order to study if CHBs harbour recurrent chromosomal changes, which may be of importance in the pathogenesis of these tumors, we performed a comparative genomic hybridization (CGH) study. We selected 22 cases of CHB, both sporadic and VHL-associated for the study. DNA was isolated from paraffin-embedded material, and the CGH study was performed using standard procedures. Chromosomal regions were interpreted as overrepresented when the ratio exceeded 1.17 (gains) or underrepresented (losses) when the ratio was less than 0.85. The most frequent finding was the loss of chromosome 6 seen in 5 cases (23%), with a minimal overlapping region at 6q. Losses on chromosome 3 were seen in 2 of the cases with concomitant loss of 6q. In one case loss on chromosome 8 was seen. No gains were detected in the tumors. The loss of chromosomal arm 6q, seen in this study and previously in other VHL-associated tumors (renal cell carcinomas and pheochromocytomas), suggest that this chromosome area may contain tumor suppressor genes involved in the tumorigenesis of VHL-associated tumors. Further studies using more refined techniques, such as fluorescence in situ hybridization and LOH should be applied to trace these genes on chromosome 6q.

Association of the PGP Transporter MDR1^{C3435T} Polymorphism With The Susceptibility To Renal Epithelial Tumors

Michael Siegsmond¹, Ulrich Brinkmann², Elke Schäffeler³, Gregor Weirich⁴, Matthias Schwab³, Michel Eichelbaum³, Peter Fritz³, Oliver Burk³, Jochen Decker⁵, Tim M Jaeger¹, Peter Alken¹, Uwe Rothenpieler¹, Reinhold Kerb², Sven Hoffmeyer², Hiltrud Brauch³

¹Department of Urology, University Hospital Mannheim, University of Heidelberg Germany; ²Epidauros Biotechnology, Bernried, Germany; ³Dr. Margarete Fischer Bosch Institute of Clinical Pharmacology Stuttgart, Germany; ⁴Institute of Pathology, Technische Universität München, Germany; ⁵Bioscientia Institute Mainz-Ingelheim, Germany

Background: Except for hereditary disease, genetic factors contributing to the development of renal epithelial tumors are unknown. There is a possibility that the MDR1 encoded plasma membrane transporter PGP influences the risk to develop renal neoplasms. PGP is known to be involved in uptake, binding, transport and distribution of xenobiotics. There is evidence that the MDR1^{C3435T} polymorphism drives expression and modulates disease risk.

Methods: In an explorative case control study we established constitutional genotype frequencies at MDR1^{C3435T} of 537 healthy control subjects and compared them to those of 212 with renal epithelial tumors patients. There were 179 clear cell renal cell carcinoma (CCRCC) and 33 tumors collectively assigned as non-CCRCC. In a second study we compared genotypes of another 150 healthy control subjects and 50 patients with three non-CCRCC types (26 papillary RCC, 11 chromophobe RCC and 13 renal oncocytic adenoma). We applied PCR-RFLP based analysis of constitutional DNA, and statistical analysis. PGP expression was analysed by quantitative immunohistochemistry.

Results: The explorative study showed a significant association between T allele frequency and the occurrence of tumors ($P = 0.007$). When tumors were histopathologically distinguished into frequent CCRCC and less frequent non-CCRCC, both patient groups contributed to this effect with a seemingly strong influence by the latter ($P = 0.0419$). The second study established the T allele as a risk factor especially for non-CCRCC ($P = 0.0005$) with the highest risk for homozygote TT allele carriers ($P < 0.0001$). Independently, we showed MDR1^{C3435T} genotype associated variations in PGP expression in normal renal parenchyma with a twofold difference of median values (TT: 0.9, CC: 1.8).

Conclusion: Our data provide evidence for PGP to influence the susceptibility to develop renal epithelial tumors by virtue of its MDR1^{C3435T} polymorphism and changes in expression. Especially T and TT carriers are at risk to develop non-CCRCC, i.e. papillary and chromophobe RCC as well as oncocytic adenomas.

VHL Alterations Associated with Good Prognosis in Sporadic Clear-cell Renal Carcinoma

Masahiro Yao¹, Minoru Yoshida¹, Takeshi Kishida¹, Noboru Nakaigawa¹, Masaya Baba¹, Kazuki Kobayashi¹, Takeshi Miura², Masatoshi Moriyama³, Yoji Nagashima⁴, Yukio Nakatani⁵, and Kei-ichi Kondo¹

¹Departments of Urology and ⁴Pathology, Yokohama City University School of Medicine, Yokohama, Japan, ²Department of Urology, Kanagawa Cancer Center, Yokohama, Japan, ³Department of Urology, Yokohama City Municipal Hospital, Yokohama, Japan, ⁵Department of Anatomical and Surgical Pathology, Yokohama City University Hospital, Yokohama, Japan

Background: Somatic alteration of the von Hippel-Lindau disease (VHL) tumor suppressor gene is one of the most common genetic changes observed in sporadic clear-cell subtype renal cell carcinoma (RCC). However, the prognostic utility of VHL mutation has not been examined. The purpose of this study was to explore the prognostic significance of VHL mutation in sporadic clear-cell renal carcinoma. **Methods:** A total of 187 clear-cell RCC patients who underwent nephrectomy from October 1986 to December 1995 were examined for somatic VHL gene alteration as well as clinicopathologic and prognostic data. **Results:** Intragenic mutation and hypermethylation of the VHL gene were found in 98 (52%) and 10 (5.3%) tumor samples, respectively. Kaplan-Meier survival plots showed that the presence of VHL alteration was strongly associated with better prognosis for stage I to III clear-cell RCCs treated by radical nephrectomy, both in terms of cancer-free and cancer-specific survival ($n=134$, log-rank: $P=.0236$ and $.0225$, respectively). These associations were statistically more significant among relatively advanced stage (stage 2+3 or stage 3 alone), up-grading (G2+3+4 or G3+4 tumors), or symptom presentation-positive cases. Cox proportional hazards modeling confirmed that the VHL alteration was an independent prognostic factor, after adjustment for the effects of sex, age, stage, grading, and symptomatic presentation. On the other hand, VHL alteration was not associated with the prognosis for patients with stage 4 tumors treated by palliative or adjunctive nephrectomy ($n=53$, log-rank: $P=.7599$).

Conclusions: presence of VHL mutation/hypermethylation was correlated with a better outcome for stage I to II clear-cell RCC patients treated by potentially curative surgery. VHL mutation analysis may be of prognostic use in sporadic clear-cell RCC.

Analysis of genotype-phenotype correlations in VHL patients with pancreatic neuroendocrine tumors.

Pascal Hammel¹, Sophie Giraud², Christophe Bérout³, Valérie Vilgrain⁴, Alain Sauvanet¹, Benoît Terris⁵, Jean-Michel Corréas⁶, Patricia Niccoli-Sire⁷, Maha Kacem⁸, Philippe Ruzsniowski¹ and Stéphane Richard^{9,10} for the French VHL Study Group.

¹Fédération Médico-chirurgicale d'Hépatogastroentérologie, ⁴Service de Radiologie, ⁵Service d'Anatomie Pathologique, Hôpital Beaujon, 92110 Clichy - ²Laboratoire de Génétique, Hôpital E. Herriot, 69437 Lyon - ³INSERM U383, ⁶Service de Radiologie, ⁹Service de Néphrologie Hôpital Necker, 75743 Paris - ⁷Service d'Endocrinologie, Hôpital de la Timone, 13915 Marseille - ¹⁰Génétique Oncologique EPHE, UPPRESS 1601 and Service d'Urologie, 94276 Le Kremlin-Bicêtre; FRANCE - ⁸Service de Médecine Interne, CHU Fattouma Bourguiba, Monastir; TUNISIE.

Background: Pancreatic neuroendocrine tumors (PNET) are a rare manifestation of von Hippel Lindau (VHL) disease but have recently emerged as potential life threatening lesions [1,2]. The aim of this study was to assess the prevalence, natural history and potential genotype-phenotype correlations in order to improve the management of PNET.

Methods: From January 1990 to December 2001, 259 consecutive VHL patients were studied in a prospective French collaborative study. All patients underwent systematic general screening for VHL including CT scan of the pancreas. Germline mutations were determined by complete gene sequencing and Southern blotting.

Results: PNET was observed in 29 patients (11.2%) with a female preponderance (19:10). Mean age at diagnosis was 33.8 ± 10 years (19-56) and PNET revealed the disease in two patients. Thirteen patients have undergone surgical resection (6 for malignant tumors), 4 patients had inextirpable metastatic tumors, one patient died because of recurrent CNS hemangioblastomas and 11 are being monitored. The 10 patients with malignant PNET (34.5%) had tumors larger than 3 cm.

Nineteen patients belonged to a VHL type 2 family and 18 had a pheochromocytoma (65.5%). VHL germline mutation was identified in 26/28 sampled patients (92.9%). Missense mutations were present in 16 cases (61.5%) and truncated protein in 10 cases. Exon 1 was involved in 6 cases, exon 2 in 2 cases and exon 3 in 15 cases. A large deletion of the *VHL* gene was present in 3 cases. A specific mutation in exon 1 resulted in an apparent risk of 50% to have a PNET.

Conclusions: PNET is highly associated but not exclusively with VHL type 2. The incidence of malignant PNET in our series is high and probably reveals the insufficiency of early detection. Analysis of *VHL* germline mutations might help to identify patients with high risk for PNET.

1 - P. Hammel et al. Gastroenterology, 2000, 119: 1087-1095.

2 - S.K. Libutti et al. Surgery, 2000, 128: 1022-8.

Somatic point mutation of the wild type allele detected in tumors of patients with VHL germline deletion

Z. Zhuang, S. Huang, S. Pack, C. A. Koch, I. A. Lubensky, E. H. Oldfield, A. O. Vortmeyer
Molecular Pathogenesis Unit, Surgical Neurology Branch NIH/NINDS

The majority of patients with VHL disease are affected by a *VHL* germline mutation involving one copy of the *VHL* gene. Loss of heterozygosity of the second *VHL* allele can be consistently demonstrated in tumor tissue from these patients suggesting that allelic deletion is a very early or even initiating event for tumorigenesis. Approximately 20% of VHL disease patients, however, exhibit germline deletion of one entire copy or at least a substantial part of the *VHL* gene. To investigate the nature of the "second genetic hit" in this patient population, we analyzed two renal cell carcinomas and one CNS hemangioblastoma from three unrelated patients for genetic changes of the second copy of the *VHL* gene.

All three tumors showed retention of one *VHL* allele by FISH. Single strand conformation polymorphism and mutation analysis of microdissected tumor DNA revealed somatic point mutations of the wild type *VHL* copies in each of the three tumors. The results indicate that the "two hit model" is equally applicable to patients with *VHL* germline mutation and *VHL* germline deletion. In contrast to tumors from patients with *VHL* germline mutation, however, point mutations of the wild-type allele can be detected in tumors from patients with *VHL* germline deletion.

Germ-line Mutations in non Syndromic Pheochromocytoma

Hartmut P.H. Neumann, M.D. Birke Bausch, Sarah R. Mcwhinney, B.A, Bernhard U. Bender, M.D., Oliver Gimm, M.D., Gerlind Franke; Ph.D., Joerg Schipper, M.D., Joachim Klisch, M.D., Carsten Althoefer, M.D., Klaus Zerres, M.D., Andrzej Januszewicz, M.D., and Charis Eng, M.D., Ph.D.,

For the Freiburg-Warsaw-Columbus Pheochromocytoma Study Group

Medizinische Universitätsklinik, Albert-Ludwigs-Universitaet, Freiburg, Germany (HPHN, BB, BUB, GF), Clinical Cancer Genetics and Human Cancer Genetics Programs, Comprehensive Cancer Center, and Division of Human Genetics, Department of Internal Medicine, The Ohio State University, Columbus, OH, USA (SM, OG, CE), Department of Otolaryngology, Albert-Ludwigs-University, Freiburg, Germany (JS), Department of Neuroradiology, Albert-Ludwigs-University, Freiburg, Germany (JK), Department of Radiology, Albert-Ludwigs-University, Freiburg, Germany (CA), Institute of Human Genetics, University of Aachen, Germany (KZ), Department of Hypertension, Institute of Cardiology, Warsaw, Poland (AJ), Cancer Research Campaign, Human Cancer Genetics Research Group, University of Cambridge, Cambridge, UK (CE)

Background The group of susceptibility genes for pheochromocytoma that included the proto-oncogene *RET* (associated with multiple endocrine neoplasia type 2 [MEN-2] and the tumor-suppressor gene *VHL* (associated with von Hippel-Lindau disease) now also encompasses the newly identified genes for succinate dehydrogenase subunit D (*SDHD*) and succinate dehydrogenase subunit B (*SDHB*), which predispose carriers to pheochromocytomas and glomus tumors. We used molecular tools to classify a large cohort of patients with pheochromocytoma with respect to the presence or absence of mutations of one of these four genes and to investigate the relevance of genetic analyses to clinical practice.

Methods Peripheral blood from unrelated, consenting registry patients with pheochromocytoma was tested for mutations of *RET*, *VHL*, *SDHD*, and *SDHB*. Clinical data at first presentation and follow-up were evaluated.

Results Among 271 patients who presented with nonsyndromic pheochromocytoma and without a family history of the disease, 66 (24 percent) were found to have mutations (mean age, 25 years; 32 men and 34 women). Of these 66, 30 had mutations of *VHL*, 13 of *RET*, 11 of *SDHD*, and 12 of *SDHB*. Younger age, multifocal tumors, and extraadrenal tumors were significantly associated with the presence of a mutation. However, among the 66 patients who were positive for mutations, only 21 had multifocal pheochromocytoma. Twenty-three (35 percent) presented after the age of 30 years, and 17 (8 percent) after the age of 40. Sixty-one (92 percent) of the patients with mutations were identified solely by molecular testing of *VHL*, *RET*, *SDHD*, and *SDHB*; these patients had no associated signs and symptoms at presentation.

Conclusions Almost one fourth of patients with apparently sporadic pheochromocytoma may be carriers of mutations; routine analysis for mutations of *RET*, *VHL*, *SDHD*, and *SDHB* is indicated to identify pheochromocytoma-associated syndromes that would otherwise be missed (N Engl J Med 2002;346:1459-66).

Fine structure mapping of chromosome 1 in sporadic and familial pheochromocytoma

G.Opocher¹, F.Schiavi¹, A.Vettori², A.Calderan², B.Vianello¹, A.Murgia³, M.Martella³, A.Taccaliti⁴, F.Mantero¹ & M.L.Mostacciolo²

¹Department of Medical and Surgical Sciences, ²Department of Biology, ³Department of Pediatrics, University of Padua, and ⁴Department of Internal Medicine, University of Ancona, Italy

The genetic mechanism underlying the tumorigenesis of pheochromocytoma has been deeply investigated but is still incompletely understood. A recent, extensive investigation on chromosomal changes in pheochromocytoma provided evidence that new tumor suppressor genes on chromosomes 1p may be involved in early tumorigenesis of pheochromocytoma. Taking advantage from this observation we have performed a systematic analysis of loss of heterozygosity (LOH) at chromosome 1

We have studied 31 highly polymorphic microsatellites regularly distributed at 4cM intervals along the entire chromosome 1, detected with fluorescent probes (ABI-Prism Linkage Mapping Set). The high-molecular-weight DNA extracted from paired peripheral blood lymphocytes and tumour tissues of 39 patients with pheochromocytoma, has been analysed: 21 were sporadic, 12 were syndromic, (5 von Hippel Lindau disease, 5 MEN 2a, 2 Type 1 neurofibromatosis) and 5 were non syndromic familial. One sporadic case had a somatic *VHL* mutation. Any sporadic case had somatic *ret* mutation.

Various sizes LOH at 1p was detected in 86 % of the sporadic cases (18/21), indicating complete to partial deletion of 1p. In 12 sporadic cases, in 4 MEN and in 2 NF1 the entire short arm was deleted. In 6 sporadic cases a partial deletion was detected while 1 sporadic case had the complete loss of chromosome 1. Except this case, 1q was never deleted. Any case with *VHL* (either constitutional or somatic) had LOH at chromosome 1. Most interestingly, 17/21 cases of sporadic pheochromocytoma share LOH at the genetic marker D1S2890 which maps at 60,6 cM from telomere and correspond to the cytogenetic band 1p32.1

In conclusion these results confirm that Chromosome 1p harbors one or more pheochromocytoma genes and suggest that one gene maps at 1p32.1. Since *SDHB*, which has been found mutated in some case of sporadic or familial pheochromocytoma maps at 1p 36.2, our results suggest the presence of one other pheochromocytoma gene in a more centromeric position.

Genotype-phenotype correlations in von Hippel-Lindau disease - the NCI experience.

Berton Zbar, Cathy Stolle, Gladys Glenn, McClellan Walther, Peter Choyke, Emily Chew, Laura Schmidt and Marston Linehan, Laboratory of Immunobiology and IRSP, SAIC Frederick, Inc., National Cancer Institute-Frederick, Frederick, Maryland USA and Urologic Oncology Branch, National Cancer Institute, Bethesda, Maryland USA

In 1998 we reported that we had identified germline VHL mutations in 93 of 93 families with clinical characteristics of von Hippel-Lindau disease. We have now identified germline VHL mutations in 245 families with clinical VHL. Using the NCI VHL classification, 74% of VHL families seen at NCI were type 1, 4% were type 2A and 22% were type 2B. We have not identified any VHL type 2C families. Missense mutations were the predominant type of mutation in VHL types 2A and 2B. VHL type 1 was associated with partial and complete deletions of the VHL gene, and point mutations that truncated the VHL gene. A database has been prepared that contains detailed clinical information on 505 patients affected with VHL evaluated at NCI. This database is being supplemented with clinical information obtained on field trips to members of VHL families. Funded in part by DHHS#NO1-CO-12400.

Detection of germline deletions in the von Hippel Lindau TUMOR suppressor gene using multiplex polymerase chain reaction of short fluorescent fragments

S. Giraud^{1,2}, S. Georges¹, S. Pinson¹, O.M. Sinilnikova¹, F. Penfornis³, D. Chauveau⁴, B. Gilbert⁵, D. Lacombe⁶, A. Dutour⁷, A. Calender¹, S. Richard^{2,4,8} and the French VHL study Group.

1- Unité de Génétique Moléculaire, Pavillon E, Hôpital E. Herriot, 69437 Lyon-FRANCE

2- Génétique Oncologique EPHE, UPRESS 1601, Faculté de Médecine Paris-Sud, 94276 le Kremlin-Bicêtre -FRANCE

3- Service d'Endocrinologie, Hôpital J. Minjoz, 25030 Besançon - FRANCE

4- Service de Néphrologie, Hôpital Necker, 75743 Paris -FRANCE

5- Département de Pédiatrie Médicale, Hôpital de Limoges, 87042 Limoges -FRANCE

6- Service de Génétique Médicale, Hôpital Pellegrin Enfants, 33076 Bordeaux - FRANCE

7- Service d'Endocrinologie -Hôpital Nord - 13915 Marseille - France

8 - Service d'Urologie-CHU de Bicêtre-94276 Le Kremlin-Bicêtre-FRANCE

Incidence of large deletions in VHL patients has been estimated to be about 20-30%. These deletions were identified by different methods: Southern blot analysis, long PCR and FISH. However, these methods are associated with several disadvantages: Southern blot analysis is DNA and time-consuming, long PCR is not efficient for detection of deletions of the whole gene and FISH misses small deletions.

We applied a semi quantitative procedure based on the multiplex PCR of short fluorescent fragments, method previously described for detection of large deletions of *MMR*, *BRCA1* and *Rb gene* (F. Charbonnier & al, Cancer Res 60, 2000). This method is based on multiplex amplification with fluorescent primers of the three exons of the *VHL* gene and a fragment of control gene, a limited number of PCR cycles, then electrophoresis and comparative analysis on ABI377 sequencer.

This method is able to detect deletions of one exon or the whole gene and is also expected to be efficient for detection of duplications, though this latter type of rearrangements has not yet been described in VHL patients. Here we present our results on a series of about 20 VHL patients without mutations searched for sequencing analysis. We have identified partial and total deletions of *VHL* gene and characterised them at genomic level.

Contribution of sporadic cases to the prevalence of Von Hippel-Lindau (VHL) gene germline mutations in the Netherlands

F.J. Hes, A.M.W. van den Ouweland, R.A. Zewald, A.L.W. Hesseling-Janssen, M. Doeleman, H.J. Eussen, D.J.J. Halley, C.J.M. Lips, J.K. Ploos van Amstel, P.L. Pearson, D.F. Majoer-Krakauer, R.B. van der Luijt.

From the Departments of Medical Genetics (FJH, RAZ, MD, JKPvA, PLP, RBL) and Internal Medicine (FJH, CJML), University Medical Centre Utrecht, PO Box 85090, 3508 AB Utrecht, the Netherlands.

Clinical Genetics (AMWO, ALWH-J, HJE, DJJH, DFM-K) University Hospital Rotterdam, the Netherlands.

Von Hippel-Lindau (VHL) disease is classically defined by the occurrence of two or more specific VHL tumours in two close relatives or within a single patient. This rigorous definition probably leads to an underestimate of the prevalence of germline mutations and particularly in the category of sporadic patients. By relaxing the diagnostic criteria for diagnosing VHL disease we have attempted to derive an estimate of the total prevalence of VHL gene germline mutations in the Netherlands.

We report germline mutations in 32 of 34 families meeting the current diagnostic criteria of VHL disease (94%) and in 10 of 22 isolated patients fulfilling VHL criteria (45%). In 88 cases not meeting VHL criteria, three germline mutations were detected (3.4%). This suggests that nonclassic cases make a relatively small but tangible contribution to the total spectrum of VHL gene germline mutations in the Netherlands. The total of 45 independent mutations is compatible with a population frequency of 1/73.000 persons.

Evidence of *de novo* mutations was found in 20% (9/45) of all germline mutations. Most sporadic cases (11 out of 12) with VHL gene germline mutations result from *de novo* mutations. Incomplete penetrance was exhibited by two independent missense mutations. Missense mutations which are incompletely penetrant can give rise to apparently sporadic situations, but with multiple mutation carriers in the family. In conclusion, nonclassic cases, *de novo* mutations and incomplete penetrance contribute to the underestimation of the frequency of VHL gene germline mutations.

Mutational Analysis of the VHL Gene: Results, Clinical Impact and Implications of a Validated Molecular Approach

Martella M¹, Polli R¹, Leonardi E¹, Finco F¹, Schiavi F², Opocher G², Murgia A¹.

¹Department of Pediatrics, ²Department of Medical and Surgical Sciences, University of Padua, Padua Italy

Molecular scanning for the detection of germline VHL gene mutations has been performed in 117 unrelated individuals referred to our centre with a diagnosis of suspected VHL disease. Our technical approach to VHL molecular testing includes quantitative and qualitative analysis for the detection of large deletion/insertion events and for the identification of frame-shift mutations and single base substitutions.

The use of this molecular scanning strategy has led to the identification of 42 different constitutional VHL gene variants.

Of these VHL variants, 27 point mutations considered disease-causing, i.e. non conservative mutations responsible of the clinical phenotype, were identified in 33 out of 33 individuals with a well documented clinical diagnosis of von Hippel-Lindau syndrome. In one case referred to us as clinical VHL we have had a negative result; the clinical picture of this individual has never been fully documented and therefore the case awaits further elucidation. Mutations were found 1 out of 13 cases of retinal haemangiomas (8%), in 2 out of 29 cases with isolated pheochromocytoma (7%) and in 4 out of 16 patients with isolated haemangioblastoma of the C.N.S. (25%). Furthermore, an intragenic variant at nucleotide 396 (P61P), already described as a rare polymorphism and an intronic alteration at nucleotide +43, in the vicinity of the splice donor site of exon 2, both were identified in individuals with sporadic neoplastic manifestations. About 32% of the disease-causing mutations we detected were represented by large-size alterations: either partial deletions (6 cases) or deletions of the entire gene (5 cases), while 68% of the pathogenic variants were 'nonsense' mutations (9%) resulting in a truncated protein and 'missense' mutations resulting in amino-acid changes. 7 new pathogenic variants have been identified. The high mutation detection rate in clinically well defined cases of VHL disease reflects the high level of sensitivity and accuracy of this technical approach and sets quality standards for VHL testing. Based on these results we can interpret the molecular data obtained in different clinical categories as truly representing the risk that individuals presenting with single VHL-related tumors be actually affected by von Hippel-Lindau syndrome and we think we can rule out the disease in many cases of uncertain clinical diagnosis.

Immunohistochemical findings of the nitric oxide synthase and p53 protein expression in renal cell carcinomas with VHL gene mutations

Mutsuo Furihata¹ M.D., Shingo Ashida² M.D., Yuji Ohtsuki¹ M.D., Taro Shuin² M.D.

¹Department of Pathology II and ²Urology, Kochi Medical School.

Molecular mechanism of contact inhibition of cell growth by tumor suppressor protein pVHL

Masaya Baba^{1,2}, Syu-ichi Hirai¹, Takeshi Kishida², Noboru Nakaigawa², Kazuki Kobayashi¹, Masahiro Yao², Yoshinobu Kubota², Shigeo Ohno¹

¹Department of Molecular Biology, Yokohama City University School of Medicine ²Department of Urology, Yokohama City University School of Medicine

One of the targets of the VHL-containing E3 enzyme is HIF- α . Therefore, it is conceivable that a decelerated breakdown of HIF- α caused by the loss of the VHL gene results in the acceleration of VEGF production and tumor angiogenesis. In fact, hyper vascularization is a typical feature of tumors in VHL diseases. However, angiogenesis itself may not be an initial event in tumorigenesis, while it is supposed to be essential for tumor outgrowth. VHL disease causes the formation of poorly vascularized cysts in various tissues as well as hyper-vascularized tumors. Therefore, VHL must also be involved directly in the growth regulation of epithelial cells. We have reported that pVHL is involved in the contact inhibition of cell growth. The growth rates of RCC cells lacking intact VHL gene and their derivatives introduced wild-type or mutant VHL expression vector did not differ significantly when they were growing in log-phase. Importantly, however, there was a difference when they reached confluency: cells lacking wild-type VHL grew continuously, while cells expressing exogenous VHL protein showed relatively limited cell growth. Using an ecdyson-inducible VHL expressing cell line, we also showed that the growth inhibition at high cell density can be released by attenuating the VHL expression. Then, we examined the molecular mechanisms of growth suppression at high cell density by pVHL. Using Gene Chip, We compared the gene expression profile of RCC cell lines infected by VHL expression adenovirus vector or empty adenovirus vector. Although almost all the genes were same at expression level without regard to VHL(+) or VHL(-), some genes differed. And these differences were remarkable at high cell density.

Regulation of intercellular Junction Assembly and Paracellular Permeability by VHL

Pilar Ávila, Miguel A. Esteban-Barragán and Manuel O. Landázuri.

Servicio de Inmunología, Hospital de la Princesa, 28006 Madrid, Spain

Hereditary or sporadic loss of the von Hippel-Lindau tumor suppressor gene (VHL) predispose affected individuals to the development of renal clear cell cancer. VHL best known function is to act as part of an ubiquitin E3 ligase complex, promoting the ubiquitination and subsequent degradation by the proteasome of the transcription factor HIF (Hypoxia Inducible Factor). VHL also plays an important role in the control of cell migration and invasiveness, through the modulation of integrin expression and the formation of cell-matrix adhesions, and reducing the secretion of extracellular proteases. We report herein, that as assessed by immunofluorescence studies, the presence of an intact VHL is required for the adequate assembly of intercellular junctions in renal cancer cells. When compared to their wild-type VHL stably transfected counterparts, different VHL(-) renal cancer cell lines present a major alteration in the distribution of the adherens junction protein beta-catenin and the tight junction protein ZO1. As expected, detergent extraction studies reveal that the interaction of these two proteins with the actin cytoskeleton is diminished in cells without VHL. Also, these phenotypic changes are functionally associated with a significant alteration of intercellular adhesion in VHL(-) cells, which leads to an increased paracellular permeability. In conclusion, our results show that VHL is required for the development of adequate intercellular junctions in renal cancer cells. We propose that these novel mechanisms may help to regulate the abnormal growth and metastasis of VHL(-) tumors.

Molecular Pathogenesis of CNS Hemangioblastoma

Gläsker S, Bender BU, Apel TW, van Velthoven V, Mulligan LM, Zentner J, Neumann HPH

Department of Neurosurgery, Freiburg University Medical Center, Department of Internal Medicine, Freiburg University Med Center, Department of Pediatrics, Queen's University, Kingston, Ontario, Canada

Cerebellar hemangioblastoma occurs sporadically or as a component tumor of autosomal dominant von Hippel-Lindau disease (VHL). Biallelic inactivation of the *VHL* tumor suppressor gene has been shown to be involved in the pathogenesis of both tumor entities. Mechanisms of *VHL* inactivation are intragenic mutations, mitotic recombination events and hypermethylation of the promoter region. The systematic and complete examination of these genetic and epigenetic phenomena in large series of VHL-related and sporadic hemangioblastomas has, thus far, not been performed.

Thus, we have investigated 29 VHL-associated and 13 sporadic hemangioblastomas for all suggested inactivating mechanisms of the *VHL* gene using Single-stranded conformational polymorphism (SSCP), Loss of Heterozygosity (LOH) and methylation analyses. Additionally, corresponding blood samples of all patients were screened for *VHL* germline mutations by SSCP and Southern blotting.

While germline mutations were identified in 94% of VHL patients, no somatic mutations of *VHL* gene were detected in any of the 29 VHL-associated tumors. Further, 62% of VHL hemangioblastomas showed LOH of chromosome 3p. Of the 13 sporadic tumors, 23% showed a single somatic mutation of the *VHL* gene that was not present in the germline. 3p LOH was identified in 50% of informative sporadic tumors. Thus, only 1/13 sporadic tumors showed inactivation of both *VHL* alleles. No VHL-related or sporadic tumor demonstrated *VHL* promoter hypermethylation.

We conclude that for the majority of VHL-related hemangioblastomas, the inactivation or loss of the *VHL* gene, as predicted by the Knudson two-hit theory, is required. However, in a subset of tumors including most sporadic hemangioblastomas, the genetic pathways involved in tumorigenesis have yet to be defined and may represent alterations of a different pathway or pathways.

Treatment policy for spinal hemangioblastoma with von Hippel-Lindau disease

Hiroshi Kanno, Isao Yamamoto, Masahiro Yao, Ryo Nishikawa, Masao Matsutani, Toshihiko Wakabayashi, Jun Yoshida, Nobuyuki Shitara, Taro Shuin

Departments of Neurosurgery and Urology, Yokohama City University; Department of Neurosurgery, Saitama Medical School; Department of Neurosurgery, Nagoya University; Department of Neurosurgery, Komagome Hospital; Department of Urology, Kochi Medical School, Japan

An approximate half of patients with von Hippel-Lindau disease (VHL) are associated with spinal hemangioblastoma which is asymptomatic or symptomatic. Asymptomatic hemangioblastomas with VHL are not always treated but often observed until symptomatic. We examined clinical features and treatment policy for spinal hemangioblastoma with VHL in Japan. Among 142 VHL cases, 66 cases (46.5%) were associated with spinal hemangioblastoma. The mean age was 34.5 years old and the ratio of male per female was 1.3. Sixty case (90.9%) were classified as VHL type 1 and 6 cases as VHL type 2. Sixty-one cases (92.4%) were associated with the other hemangioblastoma in cerebellum or brain stem while 5 cases (7.6%) were found in only spinal cord. Thirty-one (47%) cases were associated with retinal angioma and 20 cases (30.3%) with renal cell carcinoma. Twenty-eight cases (42.4%) were treated with direct surgeries whereas 38 cases (57.6%) were observed with periodic MRI. In conclusion, most of spinal hemangioblastomas with VHL are associated with the other site hemangioblastomas. Asymptomatic small hemangioblastomas with VHL should be observed with periodic MRI and direct surgery should be performed after symptomatic.

Impact of the molecular and clinical screening in Brazilian families with von Hippel-Lindau disease (VHL)JCC Rocha MD^{1,2}, AA Camargo², RLA Silva², AJG Simpson²

¹ Department of Oncogenetics, Hospital do Câncer A. C. Camargo, Sao Paulo, Brazil. ² Ludwig Institute for Cancer Research, Sao Paulo branch, Brazil.

Aim: to evaluate the impact of the molecular and clinical screening program in Brazilian families with VHL. Methods: 21 VHL families, including 3 VHL patients without family history, were included. All patients received appropriated genetic counseling. Mutation detection consisted of direct sequencing of the 3 exons of the *VHL* gene, and quantitative Southern-blot. After the identification of the deleterious mutation in the proband, the test was offered to non-symptomatic relatives to verify the carrier status. Periodic follow-up with clinical and radiological screening was performed in all individuals. Results: Germline mutations in the *VHL* gene were detected in all probands and consisted of 17 point mutations (8 new) and 4 large deletions. During the follow up (6 months to 4 years) we detected a sort of non-symptomatic associated lesions, and proper intervention was performed individually. Conclusions: Molecular diagnosis of VHL permitted the screening of non-symptomatic family members with high risk, the refinement of risk analysis with an individualized genetic counseling based on the genetic risk, and an appropriated follow up of carriers. The clinical screening program had a positive impact in the course of the disease in these Brazilian families, with a reduction of the morbidity as a result of the early diagnosis and treatment of associated lesions.

Clinical features in the VHL disease in Japan: A survey for the onset of age on the VHL associated diseases.

Taro Shuin¹, Yasuyuki Takaki¹, Masahiro Yao², Hiroshi Kanno³, Isao Yamamoto³, Ryo Nishikawa⁴, Masao Matsutani⁴, Toshihiko Wakabayashi⁵, Jun Yoshida⁵, Nobuyuki Shitara⁶

¹Department of Urology, Kochi Medical School, Kochi Japan ²Department of Urology and ³Neurosurgery, Department of Neurosurgery Saitama Medical School, Saitama Japan. ⁴Department of Neurosurgery Nagoya University School of Medicine, Nagoya Japan, ⁶Toritsu Komagome Hospital, Tokyo, Japan.

Information for VHL disease, including the numbers of family, incidence, and characteristics of VHL associated tumors are not clarified yet in Japan. To make more accurate information for this disease, we have performed a series of survey studies. We analyzed the onset age from a total number of 180 families, which are supposed to be all of the VHL families in Japan. The mean onset age for CNS hemangioblastoma(HB), retinal angioma (RA), renal cell carcinoma(RCC) and pheochromocytoma (pheo) is 27.9, 25.9, 34.5, or 30.2 years, respectively. Peak of the onset age in CNS HB, RA, RCC is 20 to 30, 20, or 25 to 35 years, respectively. The onset age in RCC is earlier than that in European countries. The onset age in RA and pheo is higher than that in European countries. Since the frequency in each type of type of germline mutation in Japan is not so much different from those in European countries, the difference in onset age between Japan and European countries may depend on the customs in each country. This information makes presymptomatic clinical diagnosis much easier and promote better prognosis for this disease in Japan.

A Register Approach to the Management of Von Hippel Lindau Disease.

Carol Giblin, Fiona Lalloo, Gareth Evans
Academic Unit of Medical Genetics and Regional Genetic Service
St Mary's Hospital, Manchester, UK

The care of families with Von Hippel Lindau Disease in the Northwest Region of England is coordinated by a Genetic Family Register Approach. The aim of the service is to :

- * Ensure families have yearly contact from the Genetics Department.
- * Organise screening and appointments for the patients who have chosen to have their screening coordinated by the Genetics department.
- * Ensure appropriate screening continues in the district hospitals for patients who prefer this.
- * Offer support and information during pregnancy.
- * Keep families up to date about advances in research.
- * Ensure that other family members are offered genetic counselling and screening.
- * Provide open access to a genetic counsellor and the department at any time between appointments.
- * Offer home visiting support service as appropriate.

There are currently 42 families on the North West Genetic Family Register.

This paper will outline how the register approach works in practice. It will highlight the need for a multidisciplinary approach and continuity of care in the management of the condition. It will describe the experiences of the families and the experience of professionals working with the families. Themes that have arisen in genetic counselling will be considered including adapting to annual screening, testing children and living with future uncertainty.

Hippel-Lindau disease in Hungary.

Pfliegler G.¹, Fazakas F.², Balázs E.³, Nagy V.³, Berta A.³.

¹Division of Rare Diseases, ²Department of Medicine, ²Department of Clinical Biochemistry and Molecular Pathology, ³Department of Ophthalmology, Center for Health and Medical Sciences, University of Debrecen,

The Hungarian Society for Hippel – Lindau disease (VHL) was founded 1998. Because VHL crosses so many medical specialities the follow of patients is now guided by the newly founded Division of Rare Diseases of University of Debrecen. A thorough clinical examination including NMR, funduscopy, US, etc. has been done in each case and beyond this a thrombophilia screening has also been carried out. In the past for years we managed to carry out a detailed genetic analysis of 8 patients and their relatives (25 altogether) by direct DNA sequence analysis. Out of the 6 patients with clinical signs at the time of genetic analysis 5 had mutations. In two of them these were novel: g8758→A (Y185X), and N78YgA46→T, respectively. In two patients gC8786→T (Q195X) and G1055+1→A variants were found. In the asymptomatic father of this latter patient the same mutation could be detected. Two years after he developed clear cell renal carcinoma which finding emphasizes the importance of thorough follow up in family members without an overt disease but mutation. Since one essential feature of VHL is hemangioblastoma and its severity is influenced by environmental and genetic modifiers the finding that inherited thrombophilic conditions (e.g. FVLeiden mutation) occurred in some cases might have a pathogenic role.

Description of the von Hippel-Lindau disease in three spanish families

Karina Villar Gómez de las Heras

General Practitioner, member of the VHL Spanish group, Centro de .Salud de Navahermosa. Toledo, Spain

PURPOSE: To describe disease evolution and clinical follow-up of 10 patients in three Spanish families. **PATIENTS AND METHODS:** Patients were contacted through the von Hippel-Lindau Family Alliance (VHLFA). Compilation of medical reports and patient interviews were carried out in May of 2001. This is a retrospective study of a case series. **RESULTS:** Family history was suggestive of von Hippel-Lindau disease in two of the families. A 'de novo' mutation is described in the third family. The mean patient age is 30,8 (range: 15-53). The age of presentation was <30 in 9 patients. In 8 of the patients, eye involvement was the first VHL presentation. 7 patients currently have hemangioblastomas of the CNS. Most patients are not participants in management protocols for the coordinate care of their various VHL lesions. 6 patients had documented pancreatic involvement and 3 patients had renal cancer. Clinical screening for occult pheochromocytomas were performed in 5 patients. 4 were deceased from VHL-related complications. Genetic studies had been performed in 4 of the 6 survivors. **CONCLUSIONS:** Disease progression in our series is similar to that previously described for other families. Diagnosis was delayed in two of the families. Suboptimal patient management may have contributed to the high morbidity and mortality. **KEY WORDS:** von Hippel-Lindau disease; hemangioblastoma, pheochromocytoma; carcinoma, renal cell.

Partial nephrectomy: alternative treatment for renal cell carcinoma in patients with Von Hippel-Lindau disease

RJA van Moorselaar¹, FJ Hes², R Lazarov¹, CJM Lips²
Departments of Urology¹ and Endocrinology²
University Medical Center Utrecht, The Netherlands

Patients with Von Hippel-Lindau disease (VHL) have an autosomal, dominantly inherited genetic defect on chromosome 3p25, predisposing them to various tumours. The incidence of renal cell carcinoma in patients with VHL disease is 24% to 45%. We have evaluated our results of nephron-sparing surgery (NSS) in VHL patients.

Material and Methods: Between 1993 and 2001 7 patients underwent 12 NSS, eight (67%) were performed with hypothermia and renal ischemia. Preoperatively a CT scan was made. Since 1998 NSS is performed when a tumour size reaches 3 cm. Mean patient age was 35.7 years (range 28-48). Either enucleation for small tumours or cysts or partial nephrectomy, i.e. removal of lesion with a 0.5 cm rim of normal tissue, was done. In one patient bench-surgery was performed. Four patients required additional surgery for cerebellar and other CNS hemangioblastomas, two laser treatments of retinal angiomas, and one bilateral adrenalectomy for pheochromocytomas. Patients were followed for recurrence by ultrasound.

Results: In total 27 tumours (range 1-9 per patient) and 8 cysts were removed. Tumour size varied from 1.9-7.5 cm. Mean cold renal ischemic time was 20 minutes (range 8-45). Except for one pT3a and one pT4 all tumours were pT1. Six patients are alive after a mean follow-up of 62 months (6-93). Mean preoperative serum creatinine was 77.8 µmol/l (range 63-105) and mean postoperative creatinine was 83.5 µmol/l (range 62-107). Two patients, 3 kidneys, had a recurrent tumour for which another NSS was performed after respectively 2, 4.5 and 5 years. Neither of the patients required dialysis nor developed metastases. One patient with a pT4 tumour died 2 months postoperatively because of a massive bleeding.

Conclusions: NSS in patients with VHL disease is an effective therapeutic option to maximize renal function while minimizing the risk of metastatic disease. The current threshold for operation is a tumour size of 3 cm.

Caring for Families with VHL – the experiences of Guy’s Hospital, London

S. Watts, F. Kavalier, S. Hodgson
Clinical Genetics, 7th Floor New Guy’s House, Guy’s Hospital,
London SE1 9RT, England

The clinical management of families with von Hippel Lindau Disease is difficult due to the variable expression of the disease. A review in June 1997 at Guy’s Hospital identified 30 VHL families, 11 of which were receiving regular follow-up. These families comprised 11 affected individuals, 26 ‘at risk’ adult relatives and 7 children. In total, 119 different screening tests were required for these individuals per year. A number of practical problems arise from such screening requirements:

- co-ordination of screening appointments,
- obtaining and recording of results and
- interpretation of results.

To provide optimum patient care and to improve communication and collaboration (both research and service) between clinicians a one-stop screening clinic, held 3 monthly, was established in January 1998. The disciplines represented at the clinic are Clinical Genetics, Neurology, Ophthalmology and Renal Medicine.

Currently 39 families attend this clinic, comprising 34 affected adults, 24 unaffected ‘at risk’ adults and 14 children. Referrals to the clinic are increasing as patients contact relatives and clinicians from across the South East Region of England become aware of its existence.

A computerised clinical register of VHL patients has recently been established. This records both individual and family data, including DNA mutation search results and outcomes of screening tests. Data from the register will be presented to illustrate the experiences of caring for VHL patients at Guy’s Hospital.

Moleculargenetic testing for VHL in vascular retinal tumors

Kreusel K.-M., N.E. Bechrakis, H.P.H. Neumann, M.H. Foerster

Augenklinik, Klinikum Benjamin Franklin, Hindenburgdamm 30, D-12200 Berlin, Germany

Background: Molecular genetic methods provide a powerful tool to test for a mutation of the VHL gene. A sensitivity of almost one hundred percent has been achieved for confirmation of VHL disease. This allows to use molecular genetics to screen for underlying VHL in case of a retinal lesion suspicious for capillary retinal angioma (RA), the ocular manifestation of VHL. Methods: We reviewed our series of 58 patients with vascular retinal lesions, which have been tested for a VHL mutation. The lesions were classified by ophthalmoscopy and fluorescein angiography as 'typical RA' (42 unrelated patients) or as 'not typical, but RA is a possible differential diagnosis' (16 patients). Ophthalmologic classification was compared to the results of moleculargenetic testing. Results: 26 patients showed multiple typical RA and 16 individuals solitary typical RA. Moleculargenetics revealed underlying VHL disease in all patients with multiple typical RA and in 44 % of patients with a single RA. In the group of patients with retinal vascular lesions not typical for RA the suspected ophthalmoscopic diagnosis was M. Coats in two patients, vasoproliferative tumor of the retina in 9 patients or unclassified vascular lesion in 5 patients. In case of a vascular lesions not typical for RA in no patient a VHL mutation was detected. Conclusions: In case of multiple typical capillary retinal angioma VHL disease is present and can be confirmed genetically. In case of a single typical capillary retinal angioma a VHL mutation may be present in about half of the patients. In case of vascular retinal lesions untypical for RA however, molecular genetic testing for VHL disease is not informative.

Retinal hemangioma in the von Hippel-Lindau (VHL) disease. Identification, evaluation and management. A review

S.Piermarocchi, G. Lo Giudice, L. Caretti, E. Pilotto.

Department of Ophthalmology, University of Padova, Padova, Italy

Background: Retinal capillary hemangioma (RH) is the most frequent and the earliest manifestation of VHL disease and therefore, an ophthalmologist is frequently involved in the care of patients with VHL. Depending on the clinical circumstance, RH may be managed by observation, laser photocoagulation, cryotherapy, plaque therapy and vitreoretinal surgery. We review herein the ophthalmic features and clinical management of RH in patients with VHL disease referred to our Department of Ophthalmology. Methods: We describe our series of 21 patients (13 females, 8 males; mean age: 24.5 years) with RH, which have been tested for a VHL mutation. An ophthalmoscopic examination and a fluorescein and ICG green angiography was carried out in all patients. Various treatment approaches have been performed about the ophthalmic findings of RH. Results: Ophthalmoscopy showed 6 patients (29%; 4 females, 2 males) with multiple retinal capillary hemangiomas and 15 patients (9 females, 6 males) with a solitary typical RH (71%), located in the peripheral retina. A half of the patients has bilateral involvement. In 1 patient the ophthalmoscopic examination disclosed in right eye a small, whitish, dome-shaped lesion scattered in the deeper layer of the posterior retina and the angiographic evaluation showed multiple window-defects during the early phase of the angiogram. Retinal hemangioma observation was performed in 13 patients (mean size 1 mm); laser photocoagulation and/or cryotherapy was applied to 5 patients (mean size 2.8 mm) and 2 patients (mean size up to 3.5 mm) respectively. One patient underwent vitreoretinal surgery and cryotherapy for multiple RHs complicated by tractional retinal detachment and macular hole. Conclusions: Retinal capillary hemangioma is commonly a solitary tumor. Approximately one-third of patients have multiple RH. Fluorescein and ICG angiography are important to analyze the blood dynamics within the tumor. Size, location and other lesions associated to the RH lead to the decision whether to treat or not.

Moleculargenetic and clinical investigation of mutations in the *SDHB* Gene

Birke Bausch¹, Sarah McWhinney, BA⁴, Bettina Bohnert-Iwan¹, Gerlind Franke, PhD¹, Mariola Peczkowska, MD.³, Joerg Schipper, MD.², Andrzej Januszewicz, MD³, Charis Eng, MD, PhD⁴, Hartmut P.H. Neumann, MD¹
Medizinische Universitätsklinik, Albert-Ludwigs-Universität, Freiburg¹, Germany, Dept. of Otolaryngology, Albert-Ludwigs-University, Freiburg², Germany, Dept. of Hypertension, Institute of Cardiology, Warsaw, Poland³, Clinical Cancer Genetics and Human Cancer Genetic Programs, Comprehensive Cancer Center, Division of Human Genetics, Dept. of Internal Medicine, The Ohio State University, Columbus, OH, USA⁴

Introduction: Susceptibility genes for pheochromocytomas include *RET*, *VHL*, *NFI* and *SDHD*. Recently Astuti et al. (*Am. J. Hum. Genet.* 2001) reported of familial pheochromocytoma and paraganglioma associated with germline mutations of the *SDHB* gene (succinate dehydrogenase subunit B). We subsequently analysed pheochromocytoma patients who are negative for mutations in the *SDHD*, *RET* and *VHL* genes and are clinically not suffering from neurofibromatosis type 1.

Methods: 271 registry based cases of apparently sporadic pheochromocytomas are investigated for germline mutations of the *SDHB* gene. We invited relatives of mutation positive subjects for genetic screening. Blood DNA was analysed by PCR and SSCP of all exons of the *SDHB* gene. Aberrant bands were sequenced. We reviewed the history of all carriers for symptomatic tumors of the adrenal glands and paraganglia.

Results: Germline *SDHB* mutations have been detected in 12 cases or a 4% frequency. There are 9 different mutations. They are located in exons 2, 4, 6 and 7. The spectrum of mutations included 5 missense, 2 stopcodon and 2 frameshift mutations. So far 13 relatives agreed to genetic testing, resulting in detection of 8 additional carriers including 3 mothers; pedigree informations are in good agreement with autosomal dominant transmission. No de novo mutations occurred in this series. Symptomatic extraabdominal tumors, e.g. an unilateral glomus tumor of the carotid body occurred in only one subject.

Conclusion: A significant proportion of patients with apparently sporadic pheochromocytomas has germline mutations of the *SDHB* gene. Symptomatic glomus tumors are seemingly rare in such patients.

¹⁸F-Fluoro-DOPA whole-body positron emission tomography for detection of pheochromocytomas: initial results

Stefan Hoegerle¹, M.D., Egbert Nitzsche¹, M.D., Carsten Althoefer², M.D., Nadir Ghane², M.D., Tanja Manz³, M.D., Ingo Brink¹, M.D., Martin Reincke⁴, M.D., Ernst Moser¹, M.D., Ph.D., Hartmut P.H. Neumann³, M.D.

Divisions of Nuclear Medicine¹ and Diagnostic Radiology², Department of Radiology, and Division of Nephrology and Hypertension³ and Division of Endocrinology⁴, Department of Internal Medicine, Albert-Ludwigs University, Freiburg, Germany

Purpose: To evaluate ¹⁸F DOPA whole-body positron emission tomography (¹⁸F DOPA PET) as biochemical imaging approach for the detection of pheochromocytomas.

Materials and Methods: ¹⁸F DOPA PET and magnetic resonance imaging (MRI) were performed in 14 consecutive patients with suspected pheochromocytomas (5 sporadic, 9 with von Hippel-Lindau disease), MIBG scintigraphy in 12 of these patients. The individual imaging procedures were assessed in consensus by specialists in nuclear medicine and radiologists blinded to the results of the other methods. The findings of the functional imaging methods were compared with MRI as reference standard. Histological verification could be obtained in 8 of 14 patients with 9 tumors.

Results: A total of 17 pheochromocytomas (11 solitary, 3 bifocal / 14 adrenal, 3 extraadrenal tumors) was detected by MRI. ¹⁸F DOPA PET and MRI showed concordant results in all 17 tumors. In contrast, MIBG scintigraphy was falsely negative in 4 patients with 3 adrenal tumors smaller than 2 cm and 1 extraadrenal tumor with a diameter of 3.6 cm. Based on these data, a sensitivity of 100% for ¹⁸F DOPA PET and 71% for MIBG scintigraphy was calculated. Specificity was 100% for both procedures.

Conclusion: The ¹⁸F DOPA PET is a highly sensitive and specific procedure for detection of pheochromocytomas and has potential as the functional imaging method of the future.

The natural history of central nervous system hemangioblastomas in von Hippel-Lindau disease (VHL)

John E. Wanebo, MD¹, Russell R. Lonser, MD¹, Gladys M. Glenn, MD, PhD⁴, Edward H. Oldfield, MD¹

Surgical Neurology Branch¹, National Institute of Neurological Diseases and Stroke, Cancer Diagnosis Branch⁴, National Cancer Institute Bethesda, MD

Object. To define the natural history and growth pattern of CNS hemangioblastomas associated with von Hippel-Lindau disease. To correlate features of hemangioblastomas that are associated with development of symptoms and need for treatment.

Methods. We reviewed serial MRI and clinical histories of 160 consecutive vHL patients with CNS tumors and serially measured volumes of tumors and associated cysts.

Results. 655 hemangioblastomas were identified in the cerebellum(250), brainstem(64, all in posterior medulla), spinal cord(331, 96% in posterior half of cord), and supratentorial brain(10). Symptoms were related to mass effect. A serial increase in hemangioblastoma size was observed in cerebellar, brainstem and spinal cord tumors as patients progressed from being asymptomatic to symptomatic and requiring surgery($p < 0.0001$). 21 of 29 symptom-producing cerebellar tumors (72%) had an associated cyst, whereas only 28 of the 221 patients(13%) without symptoms had tumor-associated cysts($p < 0.0001$). 8 of 12 symptomatic brainstem tumors had associated cysts(67%) compared to only 4 of the 52(8%) without symptoms($p < 0.0001$). By the time symptoms occurred and surgery was required the cyst was larger than the tumor causing it; cerebellar and brainstem cysts averaged 34-fold and 19-fold the size of their associated tumors at surgery. 95% of symptom-producing spinal hemangioblastomas had associated syringomyelia.

The clinical circumstance was dynamic. Among 88 patients serially imaged for = 6 months(median, 32 months), 164 of 371 hemangioblastomas (44%) and 37 of 55 tumor-associated cysts (67%) enlarged. No tumors or cysts spontaneously diminished in size. Symptomatic tumors grew at approximately 10-fold the rates of asymptomatic tumors and symptomatic cysts grew 3.2 and 1.7 times faster than the hemangioblastoma associated with them. Hemangioblastomas frequently demonstrated a pattern of growth in which they would enlarge for a period of time (growth phase) followed by a period of arrested growth (quiescent phase). Of 69 patients with documented tumor growth 18 (26%) had tumor(s) with at least two growth phases. Of the 160 patients with hemangioblastomas, 34 patients (median followup, 51 months) developed 115 new hemangioblastomas and 15 new tumor-associated cysts.

Conclusions. This study defines the natural history of CNS lesions associated with VHL. Not only were cysts commonly associated with cerebellar, brainstem, and spinal hemangioblastomas, by the time symptoms appeared the majority of the mass effect producing symptoms derived from the cyst, rather than the tumor causing the cyst. The tendency for enlargement is much greater for cysts than for hemangioblastomas. These tumors often have multiple periods of tumor growth and arrested growth. These characteristics must be considered when determining the optimal timing of screening for individual patients and for evaluating the timing and results of treatment. When defining success with irradiation therapy or anti-angiogenic therapy, stability of tumor size, a current criteria for response to therapy, may not be a reliable indicator of tumor response.

New Insights into the Histogenesis of Hemangioblastoma

A. O. Vortmeyer, K. Yuan, E. H. Oldfield, Z. Zhuang.

Molecular Pathogenesis Unit, Surgical Neurology Branch

NIH/NINDS, Bethesda, MD

Tumor suppressor gene syndromes are characterized by a multiplicity of tumors occurring in a specific set of organs. Since Knudson's two hit hypothesis, tumor suppressor gene syndromes have held great promise to elucidate the earliest events during tumorigenesis. In the VHL tumor suppressor gene syndrome, VHL disease, CNS and kidneys are consistently affected by multiple tumors, hemangioblastomas and renal cell carcinomas. Among all hereditary tumors, CNS hemangioblastomas have been the most mysterious ones, because they are composed of cells that are of unknown histogenetic origin. After performing a detailed morphologic analysis of autopsy CNS tissues, combined with genetic and immunophenotypic analysis, we demonstrate that hemangioblastomas of the CNS are far more frequent in the setting of VHL disease than previously known. Most hemangioblastomas appear to originate in nerve root tissue. Combined morphologic, genetic and immunocytochemical analysis of "in-situ" hemangioblastomas may provide new insights into the histogenesis of these tumors.

Germline mutation of the SDHB gene associated with pheochromocytoma of urinary bladder.

Mariola Pczkowska¹, Neumann HPH², Kabat M¹, Cybulska I¹, Janaszek-Sitkowska H¹, Prejbisz A¹, Januszewicz M³, Ciela W³, Januszewicz A¹.

¹Institute of Cardiology, Warsaw, Poland. ²University of Freiburg, Germany. ³Medical University, Warsaw, Poland.

Objective: Pheochromocytoma is an unique type of secondary hypertension caused by excessive production of catecholamines by the chromaffin tumour. Approximately 90% of the tumours are located in the adrenal glands and extra-adrenal pheo occurred in approx. 10% of all cases. It has also been reported that pheo may be a part of VHL and MEN2 syndrome and coexist with other disorders.

Design and Methods and Results: The aim of study was to present a clinical course and genetic study of a case of extraadrenal pheo with a rare localisation in urinary bladder.

Case report: 19-year old male patient complained intermittent severe headache and blanching of the skin occurring shortly after micturition followed by increases in BP (from 130/80 up to 300/170 mmHg). Biochemical evaluations demonstrated elevated urinary catecholamines (CA), metoxycatecholamines (MCA) and vanillylmandelic acid. Marked increase in blood catecholamines was noted immediately after micturition as compared to the period before micturition. Ultrasonography and CT showed two solid masses: first located in left lateral wall of the bladder (3,5x5 cm) and second located close to the left common iliac vein. The patient was surgically treated and both tumours were surgically removed, the first one by partial resection of the bladder. Postoperatively the patient had no elevation in BP (especially after micturition). CA and metabolites normalised.

Molecular genetic study revealed no mutations in VHL gene and the presence of RET gene polymorphism in exon 13, codon 769 (CTT/CGT). Analysis of the SDHB gene revealed a c.721 G to A mutation with the amino acid consequence C196Y.

MRI of the neck and thorax did not show any abnormalities, including paragangliomas.

Conclusions:

Extraadrenal pheochromocytoma, not associated with germline mutations of the VHL gene, is a candidate for analysis of the SDHB gene.

VHL in Italy: what has been done and what needs to be done

Murgia A, Martella M, Schiavi F, Piermarocchi S, Lo Giudice G, Scienza R, Berlucchi S, Carollo C., Orzan E, Prayer Galletti T, Pagano F, Opocher G.

“The VHL Padova Network”

Azienda Ospedaliera e Università degli Studi di Padova

The coordinated activity of the VHL Padova Network, since its beginning in 1996, has been directed toward the achievement of two main goals, the first of which being the improvement of the level of clinical care offered to individuals affected by von Hippel-Lindau disease and to their families. The second, but not less important goal of our team was the development of a program of education among health care providers that would increase the awareness about this disease and about the need for a multidisciplinary approach to it.

In the past years 117 unrelated cases have been referred to our VHL centre. Based on molecular testing, 40 individuals have been diagnosed with von Hippel-Lindau disease while such a diagnosis has been excluded in all the other subjects. All the negative cases have been subsequently subjected to a careful clinical re-evaluation that has revealed, at least in some cases the existence of a different syndrome or clinical condition. In only one case referred to as clinical VHL reviewing of the clinical data has not been possible. All the “at risk” relatives of the VHL confirmed probands who asked to be tested underwent molecular analysis to exclude the presence of the specific disease-causing mutation. All the pre-symptomatic individuals identified were counselled and enrolled in a clinical follow-up program, according to international consensus protocols. A total of 200 individuals members of the 40 VHL families have been tested.

The subjects ascertained through our network are coming from the entire Italian territory, although mainly from the central-northern regions. A few cases have been referred for genetic testing from out of the country. Our web site has been made available on the net since 1998 and an active program of meetings has been organized within the Veneto region to divulgate our clinical program.

Both clinical collaboration and technical support have been offered to local physicians taking care of VHL patients.

We should aim at a better connection and stronger collaboration between our centre and other hospitals and clinical teams involved with VHL in Italy, and suggest the collection of all the cases of VHL disease in a national registry.

National implementation of programmatic prevention of complex hereditary tumour syndromes

C.J.M. Lips, F.J.Hes and R.B. van der Luijt

From the Departments of Internal Medicine (CJML, FJH) and Medical Genetics (RBvdL, FJH), University Medical Centre Utrecht, PO Box 85090, 3508 AB Utrecht, the Netherlands;

The management of rare and complex hereditary tumour syndromes is facing a growing number of difficulties. Medical care depends on incidental activities, interest, knowledge and efforts from individual specialists. In addition, periodic clinical monitoring and preventive medicine has a low priority within the Dutch reimbursement budget. It reveals that interruption of periodical guidance of patients induces frustration and distress among families.

A project of programmatic prevention is initiated for 5 different tumour syndromes, involving 8.500 patients and a multiple of close relatives in the Netherlands. These syndromes include Von Hippel-Lindau (VHL) disease, Multiple Endocrine Neoplasia (MEN) type I, MEN type II, Tuberous Sclerosis Complex (TSC) and neurofibromatosis (NF). Individuals with a predisposition for an inherited tumour syndrome as VHL disease are most likely to benefit from early identification of the disease (preferentially by DNA analysis) followed by periodic clinical monitoring. Potentially life-threatening tumours can be removed at an early stage and may improve both prognosis and life expectancy.

The main goal of this project is to reach a national service, which will guarantee periodic clinical monitoring, and maximal prevention of patients affected with these tumour syndromes. In our opinion, this goal can be achieved by specially trained nurses, who will coordinate multidisciplinary guidance, and organise preventive and emergency cure for these patients. These nurses will cooperate with experts in the field, specialists for social and psychological issues, patient organisations and clinical genetic centres. In addition, they are responsible for providing patients with up to date clinical information (via news letters, internet, etc.).

Conclusion: a project is initiated of problem orientated preventive health care with a central role for multidisciplinary management of diagnosis and treatment. Such national services, directed on programmatic prevention, may improve patient care for complex hereditary tumour syndromes.

Searching for the von Hippel-Lindau disease (VHL): two important differential diagnosesJCC Rocha MD^{1,2}, AA Camargo², RLA Silva², IL Cendes³, AJG Simpson²

¹ Department of Oncogenetics, Hospital do Câncer A. C. Camargo, Sao Paulo, Brazil. ² Ludwig Institute for Cancer Research, Sao Paulo branch, Brazil. Department of Genetics, UNICAMP, Sao Paulo, Brazil.

Aim: To describe two differential diagnoses of VHL disease detected in the Brazilian VHL Project. Methods: The proband of family "A" was a 55 year-old man with recent symptoms of cerebellar ataxia. The patient had a bilateral kidney carcinoma (clear cell type) at 53 and reported that two of his nine siblings (one brother and one sister) had cerebellar problems; another brother had prostate cancer. The proband of family "B" was a 13 year-old boy with "cerebellar hemangioblastoma (HB)" detected when he was 10y and on clinical surveillance since that time. The main symptoms referred by the affected individuals were headache and epilepsy. A clinical and molecular investigation was performed. Results: direct sequencing of the 3 exons of the *VHL* gene and quantitative Southern-blotting revealed no alterations. The absence of mutations in the *VHL* gene made the diagnosis of VHL improbable when a 100% rate is reached in VHL families. An intensive clinical and radiological investigation was performed. The neurological examination confirmed cerebellar ataxia in the affected individuals of family "A". The brain magnetic resonance imaging (MRI) of the proband revealed cerebellar atrophy, without mass or cysts. A diagnosis of spinocerebellar ataxia was done, and a genetic investigation is on going. During the period, the proband of family "A" developed a third tumor: a diagnosis of prostate adenocarcinoma was made after 2 years. MRI investigation of the family "B" revealed the diagnosis of familial cerebellar cavernous hemangioma.

Conclusions: the negative genetic testing permitted the diagnosis of VHL in these two families. Spinocerebellar ataxia and the familial cavernous hemangioma were shown to mask the diagnosis of VHL, and represented two important differential diagnoses.

Is endolymphatic sac tumor a major criterion for diagnosis of von Hippel Lindau disease ?

Kathlyn Marsot-Dupuch¹, Frédéric Portier², Christine Le Pajolec², Sophie George³, Sophie Giraud³, Serge Bobin², Philippe Capelle⁴, Pierre Lasjaunias¹, Gérard Benoit⁵, Stéphane Richard^{5,6}.

¹Service de Neuroradiologie, ²Service d'Otorhinolaryngologie, ³Service d'Urologie, ⁴Génétique Oncologique EPHE, UPRESS 1601, Hôpital de Bicêtre, 94276 Le Kremlin-Bicêtre. France, ⁵Laboratoire de Génétique, Hôpital E. Herriot, 69437 Lyon, France. ⁶Service de Chirurgie Digestive, Institut Mutualiste Montsouris, 75674 Paris, France.

Purpose : To illustrate a large endolymphatic sac tumor (ELST) occurring without sensorineural hearing loss or abnormal audiometric tests. To report a VHL disease discovered in a patient with an ELST associated with bilateral kidney tumors and pancreatic cysts but without any hemangioblastoma.

Material : A 38-year old patient without familial history of VHL disease was referred for a right pontocerebellar angle tumor discovered during a complete check-up for bilateral kidney tumors and pancreatic cysts. There were no CNS or retinal hemangioblastoma.

Results : The patient presented with a large hypervascular destructive temporal bone tumor. This tumor enhanced a lot and presented suggestive cystic components but inner ear structure were normal. In both CT and MR imaging, the tumor strongly suggested an ELST due to its temporal bone location in the vicinity of endolymphatic sac, its signal and vascularity. A *VHL* germline mutation was identified in exon 1 (microdeletion).

Discussion : This observation has three main interests :

1. Firstly, ELST may develop without any abnormality of audiometric tests or of vestibular function test.
2. Secondly, ELST may occur in VHL in absence of any CNS or retinal hemangioblastoma.
3. Thirdly, as the patient did not present the classical clinical criteria of VHL disease, the diagnosis was only established because a *VHL* germline mutation was identified.

Conclusion : ELST may occur in VHL patients even in case of normal auditory function. Therefore, auditory screening for a selection of candidates to focused temporal bone imaging study as proposed by the NIH in 1997 is under discussion. In addition, ELST should probably be considered as a major clinical criterion for VHL diagnosis in the same way as hemangioblastoma. Last, a search for *VHL* germline mutation in patients with hypervascular temporal bone tumor located in the area of ELST should be performed systematically.

Treatment with halofuginone results in marked growth inhibition of a VHL pheochromocytoma ex-vivo.

David J. Gross, Israel Reibstein, Lola Weiss, Shimon Slavin, Hagit Dafni, Michal Neeman and Arnon Nagler
Hadassah University Hospital, Weitzmann Institute of Science and Sheba Medical Center, Jerusalem , Rechovot and Tel-Hashomer, Israel.

Halofuginone has recently been shown to inhibit tumor progression of C6 glioma and bladder carcinoma. This anti-tumoral effect was associated with decreased tumor angiogenesis rather than a direct cytotoxic effect on the tumor cells. The anti-angiogenic action of the drug could be related to its inhibition of collagen type I synthesis, inhibition of matrix metalloproteinases (MMP's), or via both mechanisms, since both collagen synthesis and MMP activity have been shown to be involved in angiogenesis. VEGF, in addition to its effect on endothelial cell proliferation, has been shown to be a potent inducer of MMP expression. Since VHL associated tumors express high levels of VEGF, it was of interest to ascertain the potential usefulness of halofuginone for treatment of these tumors. Pheochromocytoma tissue fragments obtained at surgery from a VHL type 2a patient were propagated subcutaneously in male BALB/c nu/nu (nude) mice. For experiments, 2-3 mm tumor fragments were transplanted secondarily s. c. to nude mice . Two treatment groups received halofuginone in standard lab chow at 3 and 5 ppm; control animals received regular chow. All groups were followed for six weeks post transplantation. A marked and significant diminution of tumor size and weight was observed in the drug treated animals (>90% reduction of mean tumor volume for both the 3 ppm and 5 ppm groups). In-vivo NMR analysis of tumor blood flow in halofuginone treated animals showed a significant reduction of vascular functionality; vasodilation was also reduced, albeit non-significantly. Gelatinase assays of tumor extracts revealed a reduction of MMP-9 activity in the treatment groups. Taken together, our data indicate that therapy directed at inhibition of the VEGF effect on MMP activity curtails angiogenesis and thereby tumor growth in this model system.

Surprising effects of anti-VEGF receptor therapy in von hippel-lindau patients

Stéphane Richard^{1,2,7}, Jean-François Girmens³, Laure Croisille⁴, Jeannine Yvart⁵, Nicole Casadeval⁶, Pascal Eschwège⁷, Nozar Aghakhani⁸, Philippe David⁸, Guy Allègre¹, Paul Scigalla⁹, Olivier Hermine¹⁰, Alain Gaudric³.

¹Génétique Oncologique EPHE, UPRESS 1601; ⁴Laboratoire d'Hématologie; ⁴Service de Biophysique et Médecine Nucléaire, ⁷Service d'Urologie, ⁸Service de Neurochirurgie, Hôpital de Bicêtre, 94276 Le Kremlin-Bicêtre. France. ³Service d'Ophtalmologie, Hôpital Lariboisière, 75010 Paris. France. ⁶Laboratoire d'Hématologie, Hôtel-Dieu, 75004 Paris. France. ⁹SUGEN Inc, San Francisco, CA, USA. ²Service de Néphrologie; ¹⁰Service d'Hématologie Clinique and CNRS UMR 8603; Hôpital Necker, 75743 Paris, France.

Background: Because of the major role of pVHL in VEGF expression and the key pathway of VEGF in hemangioblastoma development, treatment with antiangiogenic drugs was recently proposed in VHL patients with untreatable or multiple CNS and/or retinal hemangioblastomas.

Methods: We performed a phase II clinical trial with the anti-VEGFR-2 receptor SU5416. Two patients with CNS hemangioblastomas and one patient with retinal hemangioblastomas were included. They were treated by intravenous administration of 145 mg/m² of SU5416 twice a week. Evaluation of tumors was performed every 3 months for CNS hemangioblastomas and monthly for retina. Safety of SU5416 was monthly assessed through physical examination and laboratory tests.

Results :

1) In the patient with retinal hemangioblastomas, the only one functional eye was the seat of multiple tumors and major cystoid macular edema (CME). Under treatment, hemangioblastomas size and number do not change but CME completely disappeared after the first month, with great improvement of visual acuity (VA) from 20/40 to 20/25. The patient was treated for 7.5 months with VA remaining around 20/25.

2) In patients with CNS hemangioblastomas, we observed a tumor progression after 6 months and the treatment was stopped.

3) A secondary paradoxical polycythemia was observed in the three VHL patients, without change in erythropoietin level and with total resolution after SU5416 was stopped.

Conclusions :

1) SU5416 seems to be very efficiency on CME, probably by capillary hyperpermeability inhibition, but not on hemangioblastomas.

2) Polycythemia has never been reported in SU5416 trials for advanced malignancies and could express a specific action on red cell precursors occurring only in VHL patients. Resolving this problem probably should be of great interest for understanding the action of SU5416 and improvement of antiangiogenic therapy in VHL. These findings could also affect inclusion of patients with pre-existing polycythemia in future trials.

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Foundation and Development of the VHL Alliance in Spain

Jesusa Martínez Gómez and Karina Villar Gómez de las Heras
members of the VHL Spanish group

Thanks to the VHLFA and Myriam Gorospe, some Spanish families got in touch, with the aim of founding an association, to support and to give information about VHL to affected people and doctors, in our country. The VHL Study Spanish Group was created in December of 2000, with the collaboration of the Cancer Research National Center and doctors of the different specialities. In June of 2001 we founded the VHL Spanish Alliance. At the moment, we are more than 40 members, among affected, relatives and friends. The Spanish Alliance is a member of FEDER (Rare Diseases Spanish Federation) and EURORDIS (European Organization for Rare Disorders), and we have the support of another charities (Spanish Association Against Cancer -AECC- and Spanish Blinds National Organization -ONCE-). Although we have now establishing our functional infrastructure, we have a website since January of 2002 (www.alianzavhl.org).

VHL in Germany. Dealing with Psychological and Social Aspects of VHL

Gerhard Alsmeier, Chair, German VHL Alliance

We found that for many of our members, the psychological and social aspects of VHL in daily life are of great importance.

We decided to focus on this topic in our newsletter and in our annual meeting in addition to the medical aspects of VHL. In order to learn what the main problems were, we organized a broad discussion process at each of our regional meetings about "the social and psychological aspects of VHL".

We collected the problems our members have in dealing with VHL. Afterwards we asked them to write down the three or four topics they personally considered most disturbing. Finally we compared the results.