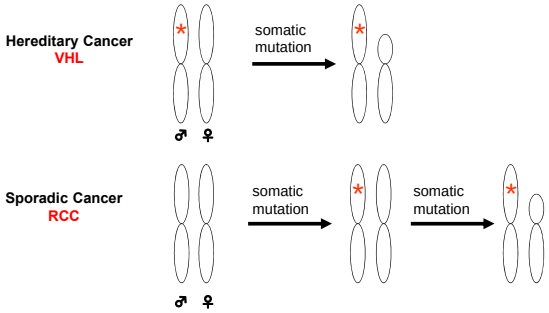
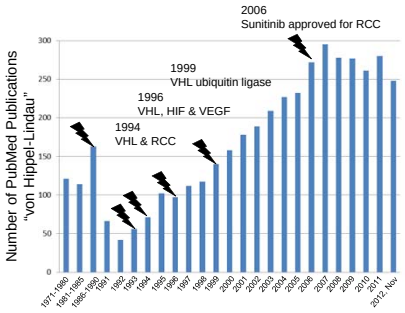
VHL Research
Where is it going?

James Gnarra, Ph.D.
Department of Urology
University of Pittsburgh Cancer Institute

research@vhl.org

VHL is a tumor suppressor gene
Knudson's 2-Hit Model for Tumorigenesis



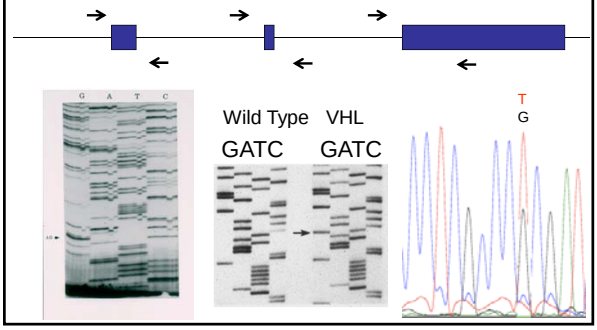


B.R. Seizinger…J.F. Gusella. von Hippel-Lindau disease maps to the region of chromosome 3 associated with renal cell carcinoma. Nature, 332: 268-269, 1988.

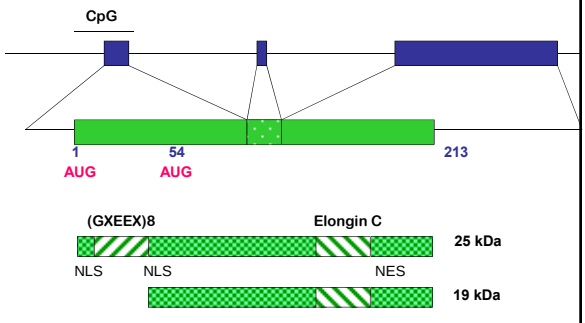
F. Latif…M. Lerman, W.M. Linehan, B. Zbar. Identification of the von Hippel-Lindau disease tumor suppressor gene. Science, 260: 1317-1320, 1993

VHL gene mutation analyses

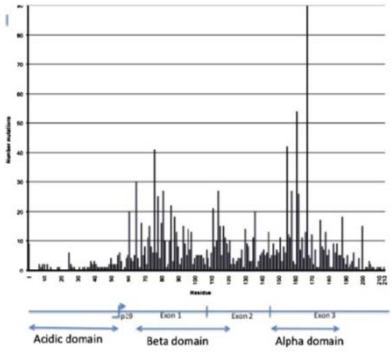
1. DNA sequencing analysis of coding exons and flanking regions
   - identifies single nucleotide or small changes



VHL Gene and Products



Organization and mutation spectrum of VHL



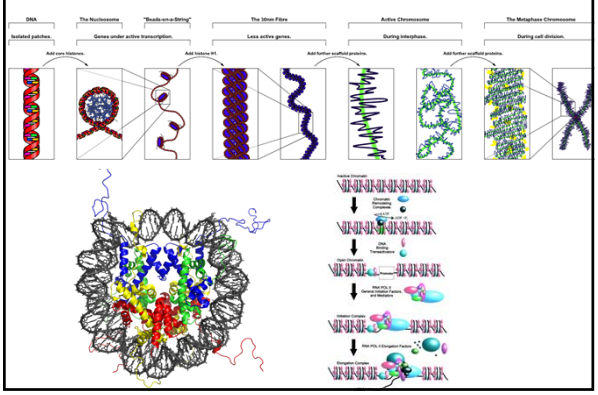
Nordstrom-O'Brien, M., et al. (2010). Genetic analysis of von Hippel-Lindau disease. Human Mutation **31**(5): 521-537.

Clinical Spectrum of von Hippel–Lindau Disease

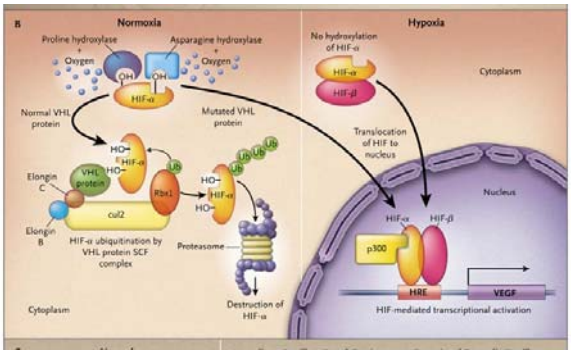
VHL subtype	VHL mutation	HB	RCC	Pheo
1	Deletion, Insertion, Nonsense, Missense	High risk	High risk	Low risk
2A	Missense	High risk	Low risk	High risk
2B	Missense	High risk	High risk	High risk
2C	Missense	Low risk	Low risk	High risk

HB, hemangioblastoma; RCC, renal cell carcinoma; Pheo, pheochromocytoma

DNA is packaged as chromatin



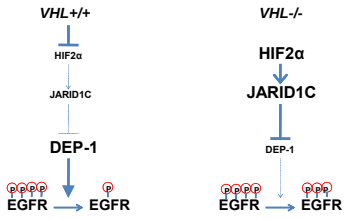
The VHL-HIF pathway



Cohen and McGovern, NEJM, 353:2477-2490, 2005

Finding:

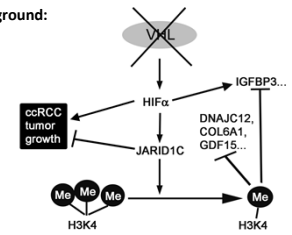
***VHL-/-* cells are more resistant to EGFR inhibitors because JARID1C represses an EGFR phosphatase, DEP-1.**



Haifeng Yang, Ph.D.
Department of Pathology
Thomas Jefferson University

Project:
Investigating the roles of histone demethylase JARID1C in RCC tumor growth and drug resistance

Background:



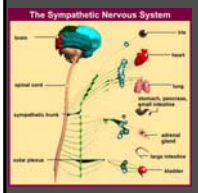
1. **In *VHL-/-* RCC cells, HIF activates H3K4Me3 demethylase JARID1C;**
2. **JARID1C reduces the global H3K4Me3 level and changes gene expressions;**
3. **HIF promotes tumor growth, while JARID1C represses it.**

Improving the molecular diagnosis of VHL disease

Eleanor Rattenberry
Prof Eamonn Maher

University of Birmingham

Pheochromocytoma and VHL disease



- *Phaeochromocytoma* catecholamine secreting tumour arising from adrenal medulla
- Paraganglioma (PGL) same tumour outside of adrenal
- Usually present with cardiovascular symptoms

- Phaeochromocytoma/PGL found in ~1% of hypertensive patients
- No known environmental, dietary, or lifestyle risk factors

- *However genetic factors are important .....*

~one third of individuals with Phaeo/PGL is genetic

20-25% of sporadic cases (no family history) may have a germline mutation

10 genes implicated in Phaeo/PGL - most often: *SDHB*/*SDHD*/***VHL***

Advantages of SGS Strategy

- Facilitates multi-gene workflows
- Increases diagnostic yield
  - Rarer genetic causes included
- Improves variant classification
- Increased potential for identifying mosaicism
- Significant reduction in cost

from ~£1250-1500 GBP for 4 gene to £500 for 9 genes

40 year old with functional PGL

SDHB

SDHD

VHL

SDHX, VHL MAX, TMEM127, RET

Why Identify Inherited Cases?

Affected individual:

- risk of multiple tumours (further phaeos)
- risk of other tumour types (VHL=haemangioblastomas, RCC; SDHB/D= HNPGL, RCC; MEN2= Medullary thyroid cancer etc)

At risk relatives:

– as above

- **To diagnose VHL disease earlier we need to be able to test *VHL* and other inherited phaeo genes more easily and be able to distinguish between disease causing and benign *VHL* variants**

What else are we doing?

- Investigating patients with variants of unknown significance in the VHL gene
  - Disease causing or not?
- Looking for 'hidden' VHL mutations in patients with VHL disease but in whom a mutation has not been found
  - Also using SGS

Birmingham Second Generation Sequencing Strategy

- **PHAEO/PGL panel:**
  - MAX
  - RET (ex10/11/13-16)
  - SDHA
  - SDHAF2
  - SDHB
  - SDHC
  - SDHD
  - TMEM127
  - **VHL**
- **SGS workflow:**
  - Target enrichment by Amplicon Fluidigm 48.48 Access Array
  - Second generation sequencing:
  - Roche GS Junior
- **Dosage:**
  - MLPA for SDHX and VHL

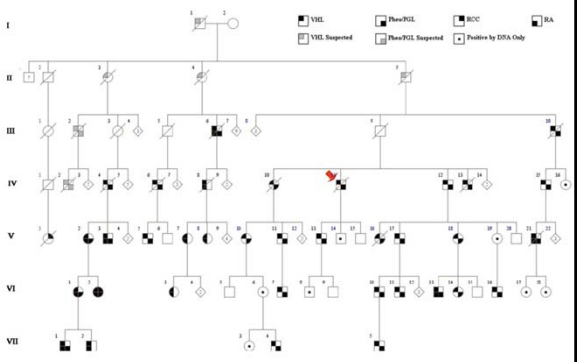
Genotype–Phenotype Correlations of Pheochromocytoma in Two Large von Hippel–Lindau (VHL) Type 2A Kindreds With Different Missense Mutations

Sarah M. Nielsen,[1] Wendy S. Rubinstein,[2,3] Darcy L. Thull,[4] Michaele J. Armstrong,[5] Eleanor Feingold,[1] Michael T. Stang,[6] James R. Gnarra,[6] and Sally E. Carty[5]*

Germany	Family 1 East Central	Family 2 Black Forest
Exon	1	1
VHL Mut.	Y112H	Y98H
Generations	7	6
Members	107	131
Pheos	65	65

	Family 1	Family 2
Patients	30	33
Mean Age	29	20
Multifocal	60%	40%
Malignant	20%	5%
Fatal	17%	0%
Extra-Adrenal	14%	28%

Family 1 (Y112H)

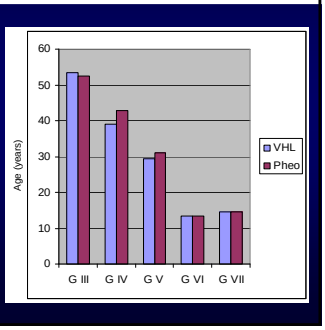


Nielsen SM, et al. AJMG, 2010.

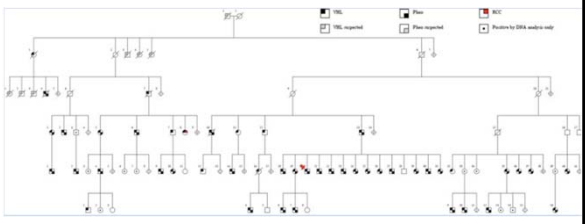
Long-term outcomes, branch-specific expressivity, and disease-related mortality in von Hippel-Lindau type 2A

Sarah M. Nielsen · Wendy S. Rubinstein · Darcy L. Thull · Michaele J. Armstrong · Eleanor Feingold · Linwah Yip · Samuel A. Tisherman · Sally E. Carty

- Patriarch came from Saxony in 1670
- Total: 11 branches w. 644 kindred members
- VHL affected: 3 branches w. 107 at risk kindred members
- Disease-related mortality decreases with generations



Family 2 pedigree (Y98H)



Branch-specific Expressivity

	Branch II-2	Branch II-3	Branch II-4
VHL Diagnosis	32%	30%	63%
Clinically Affected	78%	78%	68%
Pheo	86%	43%	100%
Pheo Only	0%	29%	81%
Retinal Angioma	86%	83%	11%
RA Only	14%	67%	0%

Nielsen SM, et al. Fam Cancer, 2011.

Comparison of 2 Large VHL Kindreds: Penetrance and Phenotype

	Members	VHL 2A	Clinically Affected	Obligate Carrier	Genetic Only
Family 1	107	46%	71%	12%	16%
Family 2	131	50%	72%	12%	15%

	Pheo	HB	RA	RCC	Panc Cyst
Family 1	70%	15%	33%	3%	3%
Family 2	66%	23%	17%	2%	0%

Nielsen SM, et al. AJMG, 2010.

Summary: Pheo Phenotype of 2 VHL kindreds

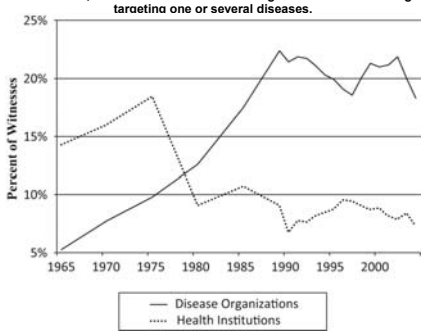
- Y112H:
  - Mean age at Pheo diagnosis is 29 years
  - Decreased mortality over time
  - Pheos are mutifocal, malignant, and mortal
- Y98H:
  - Mean age at Pheo diagnosis is 20 years
  - 80% Pheo penetrance by age 50
  - Young, unifocal, and extra-adrenal

Nielsen SM, et al. AJMG, 2010.

VHL Patient Registry

- Global database of information about VHL patients
  - Patient-entered information
- Medical History
  - number of lesions, sizes, types of scans, demographics, follow-up imaging, treatment
- Molecular mutation information
- Benefits to research
  - address questions of genetic-environmental interactions, understand development of lesions, predict response to treatment

Figure 2. Witnesses at House Appropriations Hearings Representing Disease Organizations and Health Institutions, 1965 to 2004 Note: Disease organizations includes organizations targeting one or several diseases.



Developed the viewpoint that patients are the beneficiaries of research funds and not researchers or institutions

Best R K American Sociological Review 2012;77:780-803

Disease Politics and Medical Research Funding: Three Ways Advocacy Shapes Policy

R. K. Best
American Sociological Review 77(5): 780–803, 2012

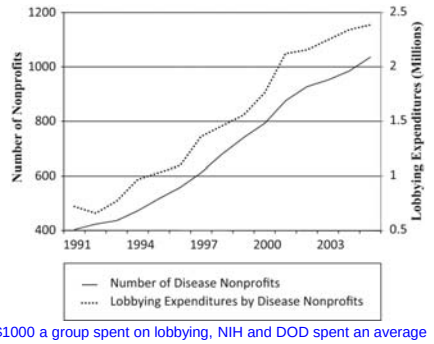
NIH Funding Shifts With Disease Lobbying, Study Suggests

J. Kaiser. Science 12 October 2012: Vol. 338 no. 6104 p. 181

H.R. 733
Recalcitrant Cancer Research Act of 2012

- Would direct the NCI to establish a scientific framework to guide research efforts on recalcitrant cancers
  - – 5 year survival rate less than 20%
  - – Death rate of at least 30,000
- Would initially choose two cancers

Figure 1. Expansion and Politicization of Disease Nonprofits, 1991 to 2004 Note: Disease nonprofits includes organizations targeting one or several diseases.



For every $1000 a group spent on lobbying, NIH and DOD spent an average of $25,000 more on that disease the following year

Best R K American Sociological Review 2012;77:780-803

Moral

Support your local VHLFA chapter and the national organization, too