The Genetics of VHL

Xia Wang MD PhD
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- Normal tissue growth is regulated by many genetic factors
- Safe traffic is maintained by traffic lights and all responsible drivers.

- How human cells and tissue grow and die?

Proper tissue growth - controlled traffic
The Genetics of VHL

Xia Wang, MD, PhD

Tampa, FL

Broken traffic light - predisposes to accident

Additional traffic light malfunction -

Reckless driving – accident!

When genetic factors are damaged with time --
The Genetics of VHL

VHL is a tumor suppressor
VHL mutations predispose to tumors
VHL affects 1 in 36,000 births

To understand genes...
- Genes are DNA molecules – genomic blueprints
- DNA → RNA
- RNA → Protein
- Proteins are the building blocks of our body

Each cell has the same set of genomic blueprints of ~20,000 genes that we are born with

DNA → RNA → Protein

VHL protein
The Genetics of VHL

DNA genetic code, amino acid, and protein

Function of VHL protein: control HIF1alpha

VHL Tumors

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Average age of onset</th>
<th>Frequency in Patients, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemangioblastoma Retina (Eye)</td>
<td>25 (0-68)</td>
<td>25-60</td>
</tr>
<tr>
<td>Cerebellum (Brain)</td>
<td>33 (9-78)</td>
<td>44-72</td>
</tr>
<tr>
<td>Brain stem</td>
<td>32 (12-46)</td>
<td>10-25</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>33 (11-66)</td>
<td>13-50</td>
</tr>
<tr>
<td>Endolymphatic sac tumor</td>
<td>22 (12-50)</td>
<td>10-25</td>
</tr>
<tr>
<td>Renal cell carcinoma or cysts</td>
<td>39 (13-70)</td>
<td>25-75</td>
</tr>
<tr>
<td>Pheochromocytoma</td>
<td>27 (5-58)</td>
<td>10-25</td>
</tr>
<tr>
<td>Pancreatic Neuroendocrine Tumor</td>
<td>36 (5-70)</td>
<td>11-17</td>
</tr>
</tbody>
</table>

Screening can find the tumor before it causes symptoms

Find the tumor early
Early intervention
Better outcomes
Screening and Frequency

<table>
<thead>
<tr>
<th>Age</th>
<th>Screening and Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>From Birth</td>
<td></td>
</tr>
<tr>
<td>0-4</td>
<td>- Physical examination and neurological assessment by pediatrician informed about VHL, using a dilated exam.</td>
</tr>
<tr>
<td></td>
<td>- Eye/retinal examination with indirect ophthalmoscope by an ophthalmologist skilled in diagnosis and management of retinal disease, especially for children known to carry the VHL mutation.</td>
</tr>
<tr>
<td>5-15</td>
<td>- Complete audiology assessment by an audiologist. Annually if any hearing loss, tinnitus, or vertigo is found.</td>
</tr>
<tr>
<td></td>
<td>- In the case of repeated ear infections, MRI with contrast of the internal auditory canal using thin slices, to check for a possible ELST.</td>
</tr>
<tr>
<td>16+</td>
<td>- Eye/retinal examination with indirect ophthalmoscope by ophthalmologist informed about VHL, using a dilated exam.</td>
</tr>
<tr>
<td></td>
<td>- Physical examination and neurological assessment by pediatrician informed about VHL, with particular attention to blood pressure taken (taken both while lying and standing), hearing issues, neurological disturbance, nystagmus, strabismus, white pupil, and other signs which might indicate a referral to a retinal specialist.</td>
</tr>
<tr>
<td></td>
<td>- Abdominal ultrasound to be started at age 8 years or earlier if indicated.</td>
</tr>
<tr>
<td></td>
<td>- Abdominal MRI or MIBG scan only if biochemical abnormalities found.</td>
</tr>
</tbody>
</table>

Who to be screened?

- If a parent has VHL, how do we know which child will be affected with VHL tumors?
Who to be screened?

- 20% of the individuals affected with VHL did not inherit it from the parents.
- The first case of VHL in the family is due to a new, i.e. de novo mutation.

VHL Inheritance Pattern – Autosomal Dominant

VHL Clinical Diagnostic Criteria

- Simplex case with two or more of the following:
  - Two or more hemangioblastomas
  - A single hemangioblastoma with multiple kidney or pancreatic cysts
  - Renal cell carcinoma
  - Pheochromocytomas
  - Endolymphatic sac tumors, papillary cystadenomas of the epididymis or broad ligament, or neuroendocrine tumors of the pancreas

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VHL diagnosis based on VHL gene testing

VHL gene structure

Human genome -- 3,000,000,000 base pairs (bps)
VHL gene – 12,633 bps, 639 bps in exons, 213 amino acids

VHL gene on the short arm of chromosome 3

Types of VHL gene changes

• Deletion of a segment of the chromosome
Types of VHL gene changes

- Base pair (DNA code) changes
- Deletion of a segment of the gene

- The technology for genetic testing has changed dramatically in the past 10 years.

Sanger sequencing reading the raw sequences of VHL gene takes 3-4 days

Large deletions detection by Southern Blot takes 4-5 days
Next-generation sequencing can detect single code change, small/large deletions or duplications. It takes 3 days to get the raw sequences of the entire human genome!

Types of VHL variants

<table>
<thead>
<tr>
<th>Classifications and Terms</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>Dysfunction</td>
</tr>
<tr>
<td>Pathological variant</td>
<td>Loss of function</td>
</tr>
<tr>
<td>Disease causing variant</td>
<td></td>
</tr>
<tr>
<td>Benign variant</td>
<td>Normal function</td>
</tr>
<tr>
<td>Polymorphism</td>
<td></td>
</tr>
<tr>
<td>Variant of uncertain significance (VUS)</td>
<td>Function unknown</td>
</tr>
</tbody>
</table>

VHL gene

Variants on VHL gene

UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly

chr3:10,141,008-10,152,220  11,213 bp  [chr3:10,141,008-10,152,220]  move   move in   move out   zoom in   zoom out zoom in  15%  10%  15%  15%  10%  15%  15%  10%  15%  15%  10%  15%  15%  10%  15%
Variants on VHL gene


One patient with Hemangioblastoma
A 2nd degree relative with Pheo

In silico prediction model

Affect intron splicing

RNA analysis

c.463+4C>T
VHL GRCh38
Chr3:10,146,640

Next gen sequencing

?? significance

Confirmed exon 2 skipping in some RNA products

Current variant classification: c.463+4C>T
VUS (variant of uncertain significance)

Samples for Genetic Testing

- Blood or Skin cells ➔ To find the germline mutation (mutation present at the beginning of life – a fertilized egg)
- Tumor tissue ➔ May reveal the germline and/or the somatic mutation (mutation occurred in later stage of life)
Genetic mutations lead to kidney cancer (Renal cell carcinoma)

By Dr. Wong Ming Ho, Edmond, Urology, HK

- Most cases of sporadic clear-cell renal carcinoma
- Mutation or silencing of VHL gene
- &
- Additional mutations

Clear-cell renal-cell carcinoma in von Hippel-Lindau disease
- Mutation, deletion, or silencing of VHL gene
- Additional mutations
- Multiple clear cell renal cysts
- Multicentric early renal tumors in cysts

Familial Erythrocytosis Type 2 (ECYT2)

- A unique condition is known in Chuvash population
- It is caused by VHL gene mutations
- But it does not cause VHL tumors

- Increased red blood cell production
- Increased levels of erythropoietin
- Normal oxygen affinity
- Thrombosis and/or hemorrhage
- Two copies of mutations on VHL, inherited in an autosomal recessive fashion.
- No individuals with one copy of the mutation have developed VHL-related tumors [Gordeuk et al 2004].
Familial Erythrocytosis Type 2 (ECYT2)

- R200W (Chuvash)
- H191D
- Russell et al. (2011)
- R200W -- Not associated with tumors seen in VHL.