Genetics of Paragangliomas and Pheochromocytomas

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Pheochromocytoma and paraganglioma (PPGLs)

Paraganglions associated with parasympathetic system

~10% metastatic dissemination

Paraganglions associated with sympathetic system

Head and neck Paraganglioma (usually non secreting)

Paraganglioma (Extra-adrenal pheochromocytoma)

Pheochromocytoma (secrating catecholamines)

Pheochromocytoma

- Rare endocrine tumour
- Annual incidence: 2-8/Million
- 0.1-0.6 % of hypertensive patients
- 5% of incidentaloma

Suggestive signs/ symptoms:
Recent, labile, refractory hypertension
Paradoxical blood pressure response
(chir, anesth, beta-blockers)
Adrenal mass
Family history of pheochromocytoma

Lenders et al., Lancet 2005; 366: 665-675
Head and neck paraganglioma
(1/30 000)

• Neural crest tumors
• Main localizations
carotid bifurcation
jugular foramen
middle ear
• Usually benign, non secreting
• Local complications and intracranial extension
• High-risk surgery in advanced forms
• Familial (30 %)
Genes associated with syndromic forms of phaeochromocytoma

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Mutations*</th>
<th>Malignancy *</th>
</tr>
</thead>
<tbody>
<tr>
<td>VHL</td>
<td>3p25-26</td>
<td>2-11 %</td>
<td>5 %</td>
</tr>
<tr>
<td>RET</td>
<td>10q11.2</td>
<td>&lt; 5 %</td>
<td>3 %</td>
</tr>
<tr>
<td>NF1</td>
<td>17q11.2</td>
<td>Unknown</td>
<td>11%</td>
</tr>
</tbody>
</table>

* in apparently sporadic phaeochromocytoma

von Hippel-Lindau (VHL)

PHE: pheochromocytoma; HAB: hemangioblastoma; RCC: renal cell carcinoma

Multiple endocrine neoplasia type 2

A: Medullary thyroid carcinoma
   Pheochromocytoma
   Hyperparathyroidism
   Cutaneous lichen amyloidosis

FMTC: Familial medullary thyroid carcinoma only

B: Medullary thyroid carcinoma
   Pheochromocytoma
   Multiple neuromas
   Marfanoid habitus

Lenders et al., Lancet 2005; 366: 665-675
Mutations of the RET Proto-Oncogene Associated with MEN-2.

Neurofibromatosis type 1: < 1% pheochromocytoma

NIH diagnostic criteria (2 or more)

- Six or more café-au-lait macules (>0.5 cm at largest diameter in a prepubertal child or >1.5 cm in post-pubertal individuals)
- Axillary freckling or freckling in inguinal regions
- Two or more neurofibromas of any type or one or more plexiform neurofibromas
- Two or more Lisch nodules (iris hamartomas)
- A distinctive osseous lesion (sphenoid wing dysplasia, long-bone dysplasia)
- An optic pathway glioma
- A first-degree relative with neurofibromatosis type 1 diagnosed by the above criteria

Hirbe and Gutmann

Rubin and Gutmann
Nature Reviews Cancer 2005: 5, 557-564
Discovery of PPGL susceptibility genes: timeline

L. Evenepoel and A. Persu
Adapted from Favier et al. *Nature Reviews Endocrinology* 2015; 11, 101–111
Mutations in SDHD, a Mitochondrial Complex II Gene, in Hereditary Paraganglioma

Bora E. Baysal,¹* Robert E. Ferrell,² Joan E. Willett-Brozick,¹ Elizabeth C. Lawrence,² David Myssiorek,⁵ Anne Bosch,⁶ Andel van der Mey,⁷ Peter E. M. Taschner,⁶ Wendy S. Rubinstein,³ Eugene N. Myers,⁴ Charles W. Richard III,⁹ Cees J. Cornelisse,⁸ Peter Devilee,⁶ B. Devlin¹

4 FEBRUARY 2000 VOL 287 SCIENCE www.sciencemag.org
SDHx subunits

Krebs Cycle

- SDHA
- SDHB
- SDHC
- SDHD

Respiratory chain

Link pheochromocytoma and \textit{SDH} genes?

Dahia PLM et al.. \textit{PloS Genet.} 2005; 1: e8
### PPGLs: main/ classical susceptibility genes

<table>
<thead>
<tr>
<th>Genes</th>
<th>Mutations</th>
<th>Malignancy</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>VHL</td>
<td>7 %</td>
<td>Low</td>
<td>AD</td>
</tr>
<tr>
<td>RET</td>
<td>6 %</td>
<td>Low</td>
<td>AD</td>
</tr>
<tr>
<td>NF1</td>
<td>3%</td>
<td>Low</td>
<td>AD</td>
</tr>
<tr>
<td>SDHB</td>
<td>10 %</td>
<td>High</td>
<td>AD</td>
</tr>
<tr>
<td>SDHD</td>
<td>9 %</td>
<td>Low</td>
<td>AD + MI</td>
</tr>
</tbody>
</table>

AD: autosomal dominant; MI: maternal imprinting

Adapted from:
SDHB mutations:
More frequent in thoraco-abdominal paraganglioma
Increased risk of recurrence and malignancy.

Modified from Pasini and Stratakis
JIM 2009; 266: 19-42
Genetic profile of sporadic head and neck paragangliomas in Belgium

Parental imprinting

Selective inactivation of one parental allele of a gene (usually by methylation)
SDHD deletion: dominant inheritance + maternal imprinting

Multiple
Rare
Localizations

Lemaire et al., JIM 1999; 246: 113-6
## PPGLs: susceptibility genes and associated phenotype

<table>
<thead>
<tr>
<th>Genes</th>
<th>Predominant tumour site</th>
<th>Tumour number (multiple versus single)</th>
<th>Family history (relative frequency)</th>
<th>Malignancy risk</th>
<th>Related conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF1</td>
<td>Pheochromocytoma &gt; paraganglioma</td>
<td>Single</td>
<td>High</td>
<td>Moderate</td>
<td>Neurofibromas, MPNSTs and gliomas</td>
</tr>
<tr>
<td>RET</td>
<td>Pheochromocytoma</td>
<td>Multiple</td>
<td>High</td>
<td>Low</td>
<td>MTC, hyperparathyroidism and marfanoid habitus</td>
</tr>
<tr>
<td>VHL</td>
<td>Pheochromocytoma &gt; paraganglioma</td>
<td>Multiple</td>
<td>High</td>
<td>Low</td>
<td>RCCs and CNS hemangioblastomas</td>
</tr>
<tr>
<td>SDHA</td>
<td>Paraganglioma</td>
<td>Single</td>
<td>Low</td>
<td>?*</td>
<td>GISTs</td>
</tr>
<tr>
<td>SDHB</td>
<td>Paraganglioma</td>
<td>Multiple</td>
<td>Low</td>
<td>High</td>
<td>GISTs and RCCs</td>
</tr>
<tr>
<td>SDHC</td>
<td>Paraganglioma</td>
<td>Multiple</td>
<td>Low</td>
<td>Low</td>
<td>GISTs</td>
</tr>
<tr>
<td>SDHD</td>
<td>Paraganglioma</td>
<td>Multiple</td>
<td>High</td>
<td>Low</td>
<td>GISTs and pituitary adenomas</td>
</tr>
<tr>
<td>SDHAF2</td>
<td>Paraganglioma</td>
<td>Multiple</td>
<td>High</td>
<td>?</td>
<td>None reported</td>
</tr>
<tr>
<td>TMEM127</td>
<td>Pheochromocytoma</td>
<td>Single</td>
<td>Moderate to low</td>
<td>Low</td>
<td>None reported. *RCC recently described, although not in association with pheochromocytoma.</td>
</tr>
<tr>
<td>MAX</td>
<td>Pheochromocytoma &gt; paraganglioma</td>
<td>Single</td>
<td>Moderate to low</td>
<td>Low</td>
<td>None reported</td>
</tr>
<tr>
<td>HIF2</td>
<td>Paraganglioma</td>
<td>Multiple</td>
<td>?</td>
<td>?</td>
<td>Polycythemia and somatostatinomas</td>
</tr>
<tr>
<td>KIF1B</td>
<td>Pheochromocytoma</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>Neuroblastoma?</td>
</tr>
<tr>
<td>PHD2</td>
<td>Paraganglioma</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>Polycythemia</td>
</tr>
<tr>
<td>HRAS</td>
<td>Pheochromocytoma</td>
<td>Single</td>
<td>?</td>
<td>?</td>
<td>None reported; gene mutated in multiple cancers†</td>
</tr>
</tbody>
</table>
TMEM 127


47 yo Belgian female with benign unilateral adrenal pheochromocytoma

Diffuse bone metastasis
Frequency of germline and somatic mutations in PPGLs

No mutation: n=81
Germline: n=78
Somatic: n=43

Relative frequency (%)
Microarray profiling

Cluster 1 → pseudohypoxia pathway

Cluster 2 → kinase signalling pathways

- Succinate accumulation
- VHL inactivation
- Stabilisation/Activation of HIF
- RET activation
- NF1 Loss of function
- RAS/RAF activation
- AKT/mTOR activation

Favier and Gimenez-Roqueplo Medsci 2012
From integrated genomics to targeted therapies

Mutation
- SDHB
- Other SDHx genes
- FH
- VHL
- NF1
- RET
- TMEM127
- MAX

Transcriptome
- Cluster 1A
- Cluster 1B
- Cluster 2

Pathways
- Hypoxia
- Angiogenesis
- Adhesion
- Glycolysis
- MAPK
- Neuroendocrine differentiation
- EMT

Methylome
- M1
- M2
- M3
- Hypermethylation
- Hypomethylation

Molecular targeted therapy
- VEGF inhibitor
- mTOR inhibitor
- TMZ
- DAC

Favier, Amar and Gimenez-Roqueplo, Nat Rev Endocrinol 2014
Genetic screening algorithm

Favier, Amar and Gimenez-Roqueplo, Nat Rev Endocrinol 2014
<table>
<thead>
<tr>
<th></th>
<th>Wild –type</th>
<th>SDHB, D or C mutation</th>
<th>SDHA mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SDHB IHC</strong></td>
<td>![Image]</td>
<td>![Image]</td>
<td></td>
</tr>
<tr>
<td><strong>SDHA IHC</strong></td>
<td>![Image]</td>
<td>![Image]</td>
<td></td>
</tr>
</tbody>
</table>

Korpershoek E.et al. *J Clin Endocrin Metab.* 2011;96(9):1472-6
A new way to do genetics: *SDHx* immunohistochemistry

**Table 2: SDHB immunohistochemistry test results according to subgroups within SDH-related and non-SDH-related tumours**

<table>
<thead>
<tr>
<th></th>
<th>Number of tumours</th>
<th>SDHB immunohistochemistry negative</th>
<th>SDHB immunohistochemistry positive</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Retrospective</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SDH-related</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDHB</td>
<td>34</td>
<td>34</td>
<td>0</td>
<td>100% (90-100)</td>
<td></td>
</tr>
<tr>
<td>SDHC</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>100% (40-100)</td>
<td></td>
</tr>
<tr>
<td>SDHD</td>
<td>38</td>
<td>38</td>
<td>0</td>
<td>100% (91-100)</td>
<td></td>
</tr>
<tr>
<td><strong>Non-SDH related</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RET</td>
<td>12</td>
<td>0</td>
<td>12</td>
<td></td>
<td>100% (74-100)</td>
</tr>
<tr>
<td>VHL</td>
<td>24</td>
<td>0</td>
<td>24</td>
<td></td>
<td>100% (86-100)</td>
</tr>
<tr>
<td>NF1</td>
<td>29</td>
<td>0</td>
<td>29</td>
<td></td>
<td>100% (88-100)</td>
</tr>
<tr>
<td>Sporadic</td>
<td>34</td>
<td>3</td>
<td>31</td>
<td></td>
<td>91% (76-98)</td>
</tr>
<tr>
<td><strong>Prospective</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SDH-related</strong></td>
<td>26</td>
<td>26</td>
<td>0</td>
<td>100% (87-100)</td>
<td></td>
</tr>
<tr>
<td><strong>Non-SDH related</strong></td>
<td>19</td>
<td>3</td>
<td>16</td>
<td></td>
<td>84% (60-97)</td>
</tr>
</tbody>
</table>


Screening strategy in the near future? (limited to operated PPGLs)

Analyse tumour by SDHB immunohistochemistry

Positive SDHB immunohistochemistry

VHL and RET genetic testing

Negative SDHB immunohistochemistry

If parasympathetic paraganglioma, SDHD genetic testing

If sympathetic paraganglioma, SDHB genetic testing

SDHB, SDHD, and SDHC genetic testing

Genetic screening of PPGLs: a pragmatic approach

Syndromic presentation: oriented genetic screening (MEN II> RET; von Hippel-Lindau> VHL)

All other PPGLs:
Screening of SDHD, SDHB, SDHC and VHL
• Exon sequencing
• MLPA (search for deletions ~ 10% of cases)

Screening of other susceptibility genes in « negative » cases, especially if early, familial, bilateral, recurrent or metastatic PPGL.

In operated patients, SDHx immunohistochemistry if available.

Near future: Next Generation Sequencing (exhaustive panel including all known susceptibility genes).

Adapted from J Vlayen (KCE), M Bex (UZ Leuven), B Bravenboer (UZ Brussel), K Claes (UZ Gent), B Lapauw (UZ Gent), A Persu (Cliniques universitaires Saint-Luc), K Poppe (CHU Saint-Pierre), U Ullman (Institut de Pathologie de Gosselies), T Van Maerken (UZ Gent), L Vroonen (Université de Liège), B Poppe (UZ Gent). Oncogenetic testing for persons with hereditary endocrine cancer syndromes (http://kce.fgov.be/fr/publication/report/tests-oncog%C3%A9n%C3%A9tiques-pour-personnes-ayant-une-pr%C3%A9disposition-h%C3%A9r%C3%A9ditaire-aux-canc).