Genética del Feocromocitoma/Paraganglioma.

Mercedes Robledo, PhD
Head of the Hereditary Endocrine Cancer Group
Human Cancer Genetics Programme
CNIO, Madrid, Spain.
mrobledo@cnio.es
Pheochromocytoma (PCC) and Paraganglioma (PGL), all together called PPGLs

Very rare: 3-8/1M

Identification of new major susceptibility genes → relevance for clinical follow-up, genetic counseling, and understanding underlying mechanisms involved in the disease.

The most inherited tumour described so far.

PPGL as example of heterogeneity
Somatic mutations: adding complexity to the mutational landscape

30-40% Somatic mutations

Other genes: IDH1, TP53, BRAF, MET, H3F3A, KMT2D...

ATRX 2015
MDH2 2015
EGLN2/PHD1 2014
FH 2013
H-RAS 2013
EPAS1/HIF2A 2012
MAX 2010
SDHA 2010
TMEM127 2009
SDHAF2/SDH5 2008
KIF16 2008
EGLN1/PHD2 2007
SDHD 2000
SDHC 2000
SDHB 2001
MEN1 1997
VHL 1993
RET 1993
NF1 1990

? 2015
So far, the genetic scenario could be summarized as:

<table>
<thead>
<tr>
<th>Major PPGL driver genes</th>
<th>In isolated families</th>
<th>In few sporadic cases</th>
<th>Reported with mutations in recognized PPGL genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF1, RET, VHL, SDHA, SDHB, SDHC, SDHD, SDHAF2, TMEM127, MAX, HRAS, EPAS1, FH</td>
<td>KIF1B, BAP1, EGLN1/PHD2, EGLN2/PHD1, MDH2, MERTK, GOT2, DMT3A, SLC23A11</td>
<td>IDH1, H3F3A, SETD2, EZH2, FGFR1, BRAF</td>
<td>ATRX, TP53, JMJD1C, KDM2B, KMT2D/MLL2, MET</td>
</tr>
</tbody>
</table>

Promoter alterations in TERT, epi-mutations in SDHC or germline MITF mutations have been recently reported.
Are all PPGL genes equally relevant?

The identification of the driver gene, the type of mutation (somatic, germline or mosaics) and therefore the deregulated pathway, can optimize clinical management strategies in terms of:

1) diagnosis of associated syndromic tumors and/or features in the patient and relatives,
2) choice of the most appropriated imaging technique,
3) assessment of clinical course and outcome,
4) treatment selection in metastatic or unresectable PPGL.
What to do in case of having patients with a single tumour?

329 index:
- 200 PCC
- 129 PGL (61 H&N, 13 T-PGL, 54 A-PGL, and 1 unknown location.)

48 germline mutations (14.6%)
- RAS
- EPAS
- RET
- VHL
- SDHB
- SDHD
- SDHC
- SDHAF2
- SDHA
- TMEM127
- MAX

281 negative

96 tumours

40 somatic mutations (41.7% of the 96 samples)

Benefit of working on tumour DNA

Any difference when considering the tumour location?

<table>
<thead>
<tr>
<th>LOCATION</th>
<th>Germline</th>
<th>P-value</th>
<th>Somatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCC</td>
<td>9</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td>PGL</td>
<td>37</td>
<td>0.0006</td>
<td>10</td>
</tr>
<tr>
<td>HN-PGL</td>
<td>15</td>
<td>0.0002</td>
<td>1</td>
</tr>
<tr>
<td>T-PGL</td>
<td>6</td>
<td>0.027</td>
<td>0</td>
</tr>
<tr>
<td>A-PGL</td>
<td>16</td>
<td>NS</td>
<td>9</td>
</tr>
</tbody>
</table>

The germline screening should be done in cases with a single tumour.
Any clue regarding age at diagnosis?

<table>
<thead>
<tr>
<th>AGE AT PRESENTATION</th>
<th>Driver mutation</th>
<th>Germline</th>
<th>Somatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pediatric (≤18) N=13</td>
<td>84.6% (11/13)</td>
<td>61.5% (8/13)</td>
<td>23.1% (3/13)</td>
</tr>
<tr>
<td>P-value</td>
<td>P=0.068</td>
<td>P=0.15</td>
<td></td>
</tr>
<tr>
<td>Adult N=131</td>
<td>58.7% (77/131)</td>
<td>29.8% (39/131)</td>
<td>27.6% (38/131)</td>
</tr>
</tbody>
</table>

→ 25% of somatic mutations, regardless of the age of diagnosis.

The somatic screening should be done even in cases with suspicious of hereditary disease.
Benefit of working with NGS

**In a syndromic presentation**
- NF1
- RET
- VHL

**Non syndromic presentation**
- Tumour location
- Metastatic disease
- Family history
- Bilateral / multiple

**Targeted NGS**

- RET, VHL, HRAS, EPAS1, NF1, SDHA, SDHB, SDHC, SDHD, SDHAF2, TMEM127, MAX, PHD2, KIF1B, FH, MDH2, DNMT3A...

**Clinical heterogeneity**: genotype is not always well predicted by phenotype.

Allow us to identify not only the potential pathogenic mutation, but also to annotate additional genetic variants that could have a role as modifier.

Are still useful genetic screening algorithms using conventional Sanger sequencing?
Results based on 423 PPGL patients recruited between 1997 and 2016 from 11 PPGL referral centers

423 index without a known mutation:
- Only germline DNA, N = 229 (54%)
- Germline and tumour DNA, N = 27 (6%)
- Only tumour DNA, N = 167 (40%)

Single → 362 (88%):
- PCC (240)
- HN-PGL (71)
- TA-PGL (49)
- Unknown-PGL (2)

Multiple → 49 (12%)
- PCC (bilateral and/or multiple) (17)
- PCC and PGL (10)
- mPGL (22)

*No data → 12

To note:
Sanger sequencing partially performed following previously proposed genetic testing algorithm → 72% of patients.

Without previous genetic studies → 118 (28%).

Aim: to know the benefit of applying targeted NGS to all PPGL patients regardless clinical features

It was possible to obtain results in 95.3% of samples (403 patients)

<table>
<thead>
<tr>
<th>Study</th>
<th>Interrogated Genes</th>
<th>Type of Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panel I</td>
<td><strong>RET</strong> (E8 to E16), <strong>VHL</strong>, <strong>NF1</strong>, <strong>MAX</strong>, <strong>TMEM127</strong>, <strong>SDHA</strong>, <strong>SDHB</strong>, <strong>SDHD</strong>, <strong>SDHC</strong>, <strong>SDHAF2</strong>, <strong>MDH2</strong>, <strong>FH</strong>, <strong>EPAS1</strong> (E9 and E12), <strong>HRAS</strong> (E2 and 3), <strong>KIF1B</strong>, <strong>MEN1</strong>, <strong>EGLN1/PHD2</strong>, <strong>EGLN2/PHD1</strong></td>
<td>Germline and <strong>frozen</strong> tumour DNA</td>
</tr>
<tr>
<td>Panel II</td>
<td><strong>RET</strong> (E8 to E16), <strong>VHL</strong>, <strong>NF1</strong>, <strong>MAX</strong>, <strong>TMEM127</strong>, <strong>SDHA</strong>, <strong>SDHB</strong>, <strong>SDHD</strong>, <strong>SDHC</strong>, <strong>SDHAF2</strong>, <strong>MDH2</strong>, <strong>FH</strong>, <strong>EPAS1</strong> (E9 and E12), <strong>HRAS</strong> (E2 and 3)</td>
<td>Tumor DNA (<strong>FFPE</strong>)</td>
</tr>
</tbody>
</table>

- Sensitivity: 99.6% (P-I) and 99.4% (P-II);
- low coverage (<50 reads) → 25 out of 381 exons (7%).

The study revealed 134 genetic variants

- 89 pathogenic mutations
- 45 VUS (42 of them found only one time):
  - 35 germline
  - 8 found in tumour DNA (2 somatic)

Proportion expected, as 95% of the patients were not syndromic and had no family history.

In fact, among those not previously studied by Sanger sequencing, the prevalence was similar to that established in non-syndromic cases (14%)

Mutations in genes that were not previously considered due to discordant or missing clinical data.
This proportion is lower than the previously described (21-24%) probably due to previous studies were done on selected cases.
1- The relevance of an appropriated material selection and 2- coincidences exist

The presence of **phenocopies**.
Targeted NGS is a promising diagnostic tool for PPGLs in comparison with the classically gene-by-gene study, but....

NGS $\rightarrow$ high number of variants of unknown significance

SDHB IHC

Expected mutations in: SDHB, SDHC, SDHD, SDHAF2

IHC SDHB

Positive

IHC SDHA

IHC SDHA

Negative

Negative

Positive

Gene to check: VHL, RET, MAX, TMEM127, FH, MDH2

MAX gene

FH gene

IHC 2SC

IHC MAX

Negative

Negative

Positive

SDHA gene

Positive

Negative

Modified from http://www.pressor.org/img/genetics2.jpg
Targeted NGS is a promising diagnostic tool for PPGLs in comparison with the classically gene-by-gene study, but...

NGS → high number of variants of unknown significance

Location and secretion

Noradrenaline
- SDHC
- SDHAF2
- SDHA
- PHD2
- FH
- MDH2
- GOT2

EpAS1**

VHL
KIF1Bß

SDHB
SDHD
SDHC
SDHA

Dopamine

MAX
TMEM127

Adrenaline
- RET
- NF1**
- H-RAS

Adrenal location

Targeted NGS is a promising diagnostic tool for PPGLs in comparison with the classically gene-by-gene study, but...

NGS → high number of **variants of unknown significance**

- To check the presence of the variant in control population (in many DB as possible)

  - There are specific-population variants.

  - There are variants that appear in low frequency in control population.

  **SDHA**
  (low penetrance)
VARIANTS FOUND IN GERMLINE:

- 6 Intronic variants
- 3 Synonymous variants
- 5 Missense variants

<table>
<thead>
<tr>
<th>VUS</th>
<th>cDNA Protein</th>
<th>dbSNP ID ExAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>V-1</td>
<td>c.8C&gt;T p.S3F</td>
<td>Not described</td>
</tr>
<tr>
<td>V-4</td>
<td>c.310A&gt;G p.R104G</td>
<td>Not described</td>
</tr>
<tr>
<td>V-7</td>
<td>c.389A&gt;G p.Q130R</td>
<td>Not described</td>
</tr>
<tr>
<td>V-8</td>
<td>c.478G&gt;A p.V160M</td>
<td>rs138541865 T: 0.01649%; 0 hom.</td>
</tr>
<tr>
<td>V-13</td>
<td>c.766G&gt;A p.A256T</td>
<td>rs147655350 T: 0.003320%; 0 hom.</td>
</tr>
</tbody>
</table>

It was needed to design functional assays for evaluating the effects of Variants of Unknown significance in MDH2, as no tumor tissue was available.

Predictors used: SIFT, Polyphen (HDIV), Polyphen (HVAR), LRT score, Mutation Taster, Mutation Assessor, fathmm-MKL, PROVEAN, MetaSVM, MetaLR

Predictors used: PoPMuSiCv33.1, ERIS, CUPSAT, I-Mutant v3.0, MAESTRO, INPS-3D

Up to five functional assays to evaluate the pathogenic character of variants

1- Immunofluorescent assay to assess post-transcriptional MDH2 localization

2- Enzymatic assay to assess the activity of the MDH2 variants

3- Molecular dynamics simulations for the assessment of conformational changes of MDH2

“To develop a curated DB of PPGL variants, with annual re-evaluation of VUS by a group of experts for purposes of reclassification and clinical guidance”
• **15-20% of patients** with apparently benign PPGLs develop metastases years after *(up to 20y)* radical resection of the primary tumor

• **Prognosis** of metastatic PPGLs is poor: 5-year survival rate = 20-60%

• **Surgery** is the current option for the disease, as there are limited treatment options

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**Aim**

Identify markers to predict metastatic disease and improve treatment
Selection of 8 miRNA for validation
miR-21-3p
miR-182-5p
miR-96-5p
miR-202-5p
miR-183-5p
miR-551b-3p

Validation of 6 miRNA
miR-21-3p
miR-182-5p
miR-96-5p
miR-202-5p
miR-183-5p
miR-551b-3p

DISCOVERY SERIES (n=443)
Sub-serie 1
(n=93)
Sub-serie 2
(n=171)
Sub-serie 3
(n=179)

VALIDATION SERIES (n=49)
Serie 4
(n=49)

miRNA profiling of PPGL

~ 100 · 10^6 people

de Cubas et al, 2013
Castro-Vega et al, 2015
Fishbein et al, 2017
miR-96-5p

miR-21-3p

miR-202-5p

miR-183-5p

miR-182-5p

miR-551b-3p

Progression free survival probability

Time (days)

0

3000

6000

9000

12000

P = 0.041

P = 0.018

P = 0.293

P = 0.0003

P = 0.0012

P = 0.0046

High expression

Low expression

Time to progression assessment

P = 0.0003

P = 0.0012

P = 0.0046
**Risk model of metastasis prediction**

- **AUC=0.837 (P=9.9x10^{-22})**
- **AUC=0.804 (P=4.7x10^{-18})**
- **AUC=0.637 (P=9.9x10^{-5})**

**Cell migration**

- **SK-N-AS**

- Reference line

- **t=0 hours**
- **t=24 hours**

**WT**

- OX miR183+21

**SDHB KD**

- **WT OX miR183+21**
- **SDHB KD OX miR183+21**

High miR-21, miR-183 and SDHB status are involved in metastases progression.
EXPLORATORY SERIES (n=38)

Series 5
(n=38 + n=10 healthy)

Healthy controls
Patients with non-metastatic disease
Patients with stable metastatic disease
Patients with progressive metastatic disease

miRNA signature in circulation

![Graph showing copies of miRNAs in serum across different conditions]
- The germline screening should be done in cases with a single tumour → at least 14% will be a carrier of a pathogenic mutation.

- The somatic screening should be done even in cases with suspicious of hereditary disease → about 25% of pediatric cases are due to a somatic alteration.

  → Genetic counselling and clinical management

  → Benefit of working with tumour DNA (germline, somatic or mosaic). Special attention to the selection of the material.

- NGS → numerous VUS, which are a challenge for clinical diagnosis.

  → Their classification is a resource- and time-demanding task (accurate clinical information, IHC, appropriated functional assays).

  → International cooperative efforts are required to update existing databases.

- Genomic information → useful source for identifying markers and select therapies.
ACKNOWLEDGEMENTS

National collaborators

International collaborators
Genomics has been useful to identify features of utility for diagnosis and follow-up.

<table>
<thead>
<tr>
<th>C1A</th>
<th>C1B</th>
<th>C2A</th>
<th>C2B</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDHB</td>
<td>SDHs</td>
<td>FH</td>
<td>MDH2</td>
</tr>
<tr>
<td>Noradrenergic + dopamine</td>
<td>Noradrenergic and adrenergic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SDHB/SDHA</th>
<th>2SC</th>
<th>MAX</th>
</tr>
</thead>
</table>

5-hydroxymethylcytosine (5-hmC)

**Norepinephrine transporter (18F-FDA-PET)**

- Glucose membrane transporter (18F-FDG PET)
- Somatostatin receptors (68Ga-DOTATATE PET)
- Succinate-pulsed proton magnetic resonance spectroscopy

**Aminoacid transporter (18F-FDOPA PET)**
It has been suggested relevant for personalized treatment