GENETICS OF PITUITARY DISEASE

Dr Ann McCormack
St Vincent’s Hospital
Garvan Institute
Sydney
Talk Outline

- Pituitary gland development
- Congenital hypopituitarism
- Hypogonadotrophic hypogonadism/Kallmann syndrome
- Central precocious puberty
- Familial pituitary tumour syndromes
- Sporadic pituitary tumours
Pituitary Gland Development

- Anterior lobe – oral ectoderm (via Rathke’s pouch)
- Posterior lobe – neural ectoderm
- Pituitary embryogenesis is complex
  - Spatio-temporally regulated signaling molecules
  - Transcription factors
Pituitary cell differentiation

Congenital Hypopituitarism

- Association with a range of midline defects
  - Holoprosencephaly
  - Cleft palate
  - Septo-optic dysplasia

- Pituitary stalk interruption syndrome – triad
  - Thin/interrupted pituitary stalk, ectopic/absent posterior pituitary and hypoplasia/aplasia anterior pituitary

- Range of endocrinopathy seen

Congenital Hypopituitarism – expanding genetic repertoire

- 5-10% have mutations in genes implicated in pituitary development or differentiation
  - PROP1 most common cause CPHD
  - Expanding repertoire of other implicated genes
    - GLI2, FGF8, LHX3, LHX4, HESX1, SOX2, SOX3, OTX2, POU1F1, TBX19, FGFR1, PROKR2
- Other associated phenotypic features may suggest a gene e.g. polydactyly (GLI2), oesophageal atresia (SOX2)

Highly variable phenotypes

FGF8 mutation
Holoprosencephaly + corpus callosum agenesis

PROP1 mutation
Anterior pituitary hypoplasia

HESX1
Identical mutation
Different phenotype

Table 1. Summary of selected clinical phenotypes and MRI findings of CPHD/SOS patients with HESX1 mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Clinical symptoms</th>
<th>Affected hormones</th>
<th>MRI findings</th>
<th>HESX1 mutation</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 1.1, and 1.2</td>
<td>Both females</td>
<td>Hydrocephalus</td>
<td>ACTH, GH, TSH, LH, FSH</td>
<td>AIP1, EPP, GON1, hypoplasia of the corpus callosum</td>
<td>p.R160C</td>
<td>6 and 9</td>
</tr>
<tr>
<td>2-1</td>
<td>Female</td>
<td>Short stature</td>
<td>GH, TSH, LH, FSH</td>
<td>AIP1, NPP</td>
<td>p.R167T</td>
<td>This study</td>
</tr>
<tr>
<td>3-2</td>
<td>Male</td>
<td>Lethargic and emaciated at 8 h after birth</td>
<td>ACTH, GH, TSH, LH, FSH</td>
<td>PA, PP is present and functioning</td>
<td>p.(R159W)/[R160H]</td>
<td>This study</td>
</tr>
</tbody>
</table>

CPHD, combined pituitary hormone deficiency; SOS, septo-optic dysplasia; GON1, optic nerve hypoplasia; ACTH, adrenocorticotropic; GH, growth hormone; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; AIP1, anterior pituitary hypoplasia; PA, pituitary aplasia; PP, posterior pituitary; SPP, optic posterior pituitary; NPP, normal posterior pituitary.

Ziemnicka K et al. J Appl Genetics 2016
Congenital hypopituitarism and Kallmann Syndrome – an emerging overlap

- May share a number of phenotypic features
  - Craniofacial defects (high-arched palate, cleft lip/palate)
  - Dental agenesis
  - Sensorineural hearing loss

- Multiple genes now recognised to cause KS or HH
  - KAL1, FGFR1, FGF8, PROKR2, PROK2, CHD7, WDR11, NELF, SEMA3A
Digenic inheritance may be an important cause of pituitary insufficiency syndromes

- Heterozygous mutations in WDR11 and PROKR2
  - both parents unaffected heterozygous carriers of each gene

- In another case of Kallmann syndrome the same PROKR2 heterozygous mutation was found in combination with an FGFR1 heterozygous mutation

Whole exome sequencing performed in a child with pituitary stalk interruption syndrome

McCormack S JCEM 2017
Sarfati et al JCEM 2010
Digenic inheritance and mutational load

- A digenic disorder results from heterozygous mutations in 2 distinct genes
  - Impact of having mutations in 2 different genes in same pathway can be more than additive, together producing a more severe phenotype
- Increasing numbers of disorders demonstrating digenic inheritance e.g. retinitis pigmentosa
- Digenic inheritance may explain incomplete penetrance
- Mutational load (particularly in cancer) refers to importance of interplay of multiple genes
Central precocious puberty

- Early activation of HPG axis (<8 girls, <9 boys)
- More common than congenital hypogonadotrophic hypogonadism yet less known about it genetically
- Rare genetic syndromes described with CPP
  - e.g. Temple syndrome (maternal uniparental disomy chromosome 14)
- Most cases CPP idiopathic and 1/3 familial
- Mutations in KISS and KISS1R described but uncommon in sporadic idiopathic CPP
- Segregation analysis - autosomal mode of inheritance but with incomplete sex-dependent penetrance

Simon D et al EJE 2016
Mutations in MKRN3 are common in familial central precocious puberty

- **MKRN3** (makorin RING-finger protein 3)
  - Maternally imprinted gene (disease only expressed in offspring of father’s carrying mutation)
  - 46% cases of familial CPP
  - Mechanism unclear but may inhibit neuronal network controlling GnRH secretion
- **DLK1** recently described causing CPP
  - also maternally imprinted

Abreu AP et al NEJM 2013
Simon D et al EJE 2016
Dauber A et al. JCEM 2017

WES study in 15 kindreds with CPP
Germline *FGFR1* Rare Variant

**FATHER**  
42 years old  
Giant prolactinoma  
(age 15)  
Visual loss, headaches  
Secondary hypopit

**DAUGHTER**  
8 years old  
Isolated GH defic  
Ant pit hypoplasia  
Ectopic post bright spot  
Strabismus
Familial Pituitary Tumour Syndromes

Charles Byrne (1761-1783)
“The Irish Giant”

The Shields Brothers
(Texas Giants)
~1880
2000
5%

SDHC
AIP
MEN1
SDHD
SDHB
SDHA
PRKAR1A
CDKN1B

Familial Pituitary Tumour Syndromes
2017+
~30%?
Pituitary Tumour Predisposition Gene Discovery Timeline

1989
- MEN1 isolated to Chromosome 11

1991
- GNAS activating mutations as cause McCune Albright syndrome

2000
- PRKAR1A mutations described in Carney Complex

2006
- AIP – first gene implicated in FIPA discovered
- p27Kip1 mutations described as cause MEN1-like phenotype

2012
- SDHx mutations first postulated to contribute to pituitary adenoma predisposition

2014
- Xq26 microduplications and GPR101 mutation described in association with XLAG syndrome
Multiple Endocrine Neoplasia Type 1 (MEN1 mutation)

- High penetrance (99% by 5th decade)
- Autosomal dominant (de novo 10%)
- 30-50% pituitary adenomas (PA)
  - Most commonly PRL
  - 15% 1st manifestation
- ~3% of “sporadic” PA (6% paediatric)
- Delay in MEN1 diagnosis 7.6-17.2 yrs
  - longest for PA presentation
- Recent MEN1 guidelines don’t suggest screening in sporadic isolated PA cases
- 70% of deaths directly attributed to by MEN1
  - Most often malignant GEP and thymic NETs

Yamazaki M Endocrine Journal 2012
Thakker R Mol Cell Endocrinol 2014
Thakker R JCEM 2012
Rare causes of PA predisposition

• **McCune Albright Syndrome**
  – Postzygotic somatic GNAS mutations with cellular mosaicism
  – Classic triad of precocious puberty, café-au-lait spots, polyostotic bone dysplasia
  – Other features can include acromegaly, thyroid adenomas, ovarian cysts

• **Carney Complex**
  – Germline PRKAR1A mutation (AD)
  – Spotty skin pigmentation, myxomas, PPNAD (Cushing’s), schwannomas, acromegaly, thyroid/breast tumours

• **MEN4**
  – Germline p27 mutation (AD)
  – 3% of MEN1 negative kindreds

*Thakker RJ Mol Cell Endocrinol 2014*
AIP and Familial Isolated Pituitary Adenoma (FIPA)

- FIPA accounts for ~2% PA cases
- AIP - first FIPA gene described
  - 20% of FIPA cases
  - tumour suppressor gene Chr 11q13
  - mechanism leading to pituitary tumourigenesis still being determined; may involve loss of inhibition cAMP synthesis
  - Disease penetrance just 30%
- Clinical characteristics \( AIP^{mut+} \)
  - Male (68%)
  - GH or PRL (84%); gigantism (36%)
  - Macroadenomas (88%)
  - 78% PA before age 30; almost all by 40
  - Treatment resistance common

*Daly AF Endo Metab Clin North Am 2015
## AIP mutation testing in sporadic PA

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>AIP positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporadic unselected</td>
<td>3-8%</td>
</tr>
<tr>
<td>Sporadic macroadenoma</td>
<td>11.7%</td>
</tr>
<tr>
<td>Sporadic macroadenoma</td>
<td>20.5%</td>
</tr>
<tr>
<td>Gigantism</td>
<td>29%</td>
</tr>
</tbody>
</table>

*Family history often not recognised*

---

Tichomirowa et al EJE 2011  
Hernandez-Ramirez JCEM 2015  
Preda V et al EJE 2014  
Rostomyan L ERC 2015
“3PAs” syndrome: PA, PGL and/or PHAEO

70 reports in literature combination acromegaly and phaeo/PGL in a patient since 1952

<table>
<thead>
<tr>
<th>Genes</th>
<th>Number of Patients With Sequence Variant</th>
<th>Sequence Variant</th>
<th>LOH in the Pituitary Adenoma</th>
<th>LOH in the Pheochromocytoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDHA</td>
<td>2 (2 variants)</td>
<td>c.969C&gt;T (p.Gly323Gly)</td>
<td>No LOH</td>
<td>Not tested</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.91C&gt;T (p.Arg31Ter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.2867C&gt;T (p.Ser100Pro)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.587G&gt;A (p.Cys196Gly)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDHB</td>
<td>9 (8 mutations and 1 variant)</td>
<td>SDHB del exons 6-8</td>
<td>3 LOH</td>
<td>Tested and identified in 1 case</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.423-1G&gt;A</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.770dupT (p.Asn258Glu/Leu17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variant: c.80G&gt;A (p.Arg27Gln)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDHC</td>
<td>2 (2 mutations)</td>
<td>c.380A&gt;G (p.His127Arg)</td>
<td>NA</td>
<td>Not tested</td>
</tr>
<tr>
<td>SDHD</td>
<td>2 (2 mutations)</td>
<td>c.242C&gt;T (p.Pro81Leu)</td>
<td>NA</td>
<td>Not tested</td>
</tr>
<tr>
<td>SDHAF2</td>
<td>1 (variant)</td>
<td>c.527&gt;C</td>
<td>NA</td>
<td>Not tested</td>
</tr>
<tr>
<td>VHL</td>
<td>2</td>
<td>c.340G&gt;C (p.Gly114Arg)</td>
<td>NA</td>
<td>Not tested</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.588G&gt;A (p.Asp197Asn)</td>
<td>NA</td>
<td>Not tested</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.1452delG (p.Thr557Ter)</td>
<td>No LOH</td>
<td>Not tested</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.783+1G&gt;A</td>
<td>Not tested</td>
<td>2 LOH</td>
</tr>
</tbody>
</table>

19 sporadic
20 familial

Denes J et al JCEM 2015
X-LAG syndrome and GPR101 overexpression

• GPR101 orphan G-protein coupled receptor
  – Found in Xq26 region
  – Potent activator of cAMP
  – Mutations → increased cell proliferation and GH expression in GH3 rat cells

• Xq26 microduplication cases
  – Predominantly female
  – All acro-gigantism <5 years old
  – Somatic mosaicism described

• 248 cases sporadic acromegaly
  – None with Xq26 microduplications
  – 11 GPR101 mutations (p.E308D)
    • 3 germline, 8 somatic

Trivellin G et al. NEJM 2014
Iacovazzo et al Acta Neuropath Comm 2016
Genetic variation permissive to PA predisposition?

<table>
<thead>
<tr>
<th>Condition</th>
<th>Gene</th>
<th>SNP</th>
<th>F PA</th>
<th>F C</th>
<th>OR (CI 95%)</th>
<th>P</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td>MEN1</td>
<td>rs29596956</td>
<td>0.024</td>
<td>0.001</td>
<td>17.8 (2.18–145.5)</td>
<td>0.0002</td>
<td>0.005</td>
</tr>
<tr>
<td>GH-secretating PA</td>
<td>SSTR5</td>
<td>rs34637914</td>
<td>0.113</td>
<td>0.040</td>
<td>2.63 (1.30–5.06)</td>
<td>0.003</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2076421</td>
<td>0.262</td>
<td>0.363</td>
<td>0.62 (0.41–0.95)</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>SSTR5</td>
<td>rs169068</td>
<td>0.554</td>
<td>0.456</td>
<td>1.48 (1.01–2.16)</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Prostate, prolactinoma</td>
<td>MEN1</td>
<td>rs624975</td>
<td>0.228</td>
<td>0.141</td>
<td>1.80 (1.06–3.06)</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>NFPA</td>
<td>DRD2</td>
<td>rs7125415</td>
<td>0.194</td>
<td>0.105</td>
<td>2.06 (1.05–4.04)</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>GH- or prolactin-secretating PA with extrasellar growth</td>
<td>DRD2</td>
<td>rs7131056</td>
<td>0.587</td>
<td>0.337</td>
<td>2.79 (1.58–4.95)</td>
<td>0.0004</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>DBD2</td>
<td>rs4938025</td>
<td>0.585</td>
<td>0.326</td>
<td>2.92 (1.60–5.30)</td>
<td>0.0004</td>
<td>0.002</td>
</tr>
</tbody>
</table>

In sporadic PA cases MULTIPLE indels and missense variants in SDHx genes – most considered benign but many ”variants of uncertain significance”

Sdhb +/- mice
Pituitary hyperplasia
Increased expression HIF1α

“preneoplastic” ?

Peculis R EJE 2016
Xekouki P et al JCEM 2015
Mutational load in PA phenotype?

Familial Pituitary Tumour Project NGS Panel

 Patients n=44

<table>
<thead>
<tr>
<th>Gene Variant</th>
<th>ExAC</th>
<th>In silico</th>
<th>Clin Var/literature</th>
<th>Clinical category</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIP p.R106C</td>
<td>3.52x10^{-5}</td>
<td>Deleterious</td>
<td>Novel</td>
<td>VUS</td>
</tr>
<tr>
<td>MEN1 p.R176Q</td>
<td>1.2x10^{-2}</td>
<td>Tolerated</td>
<td>Benign</td>
<td>Benign</td>
</tr>
</tbody>
</table>

31 year old male atypical PRLoma and FHx PHPT

2 rare variants in 2 patients

deSousa et al. EJE 2017
Mixed familial phenotypes

Son*: PHPT, MTC, PHAEO
Father: PIT (ACTH), PHPT, MTC, PHAEO
Son*: PIT (PRL), PTC
Mother: ACC
Maternal aunt: PIT (NFA)
1º Cousin: PTC

Phenocopy
RET mutation = MEN2A

?other genes
Germline CDH23 mutations in familial and sporadic PAs

- Heterozygous loss-of-function CDH23 mutation (c.4136G>T)
  - Found in 4 affected and 2 unaffected (reduced penetrance?)
- Further CDH23 heterozygous mutations in 3/12 FIPA families AND 15/125 (12%) sporadic PAs (range of subtypes)
- CDH23 - member of cadherin superfamily involved in cell-cell adhesion
- Germline mutations in CDH23 also found in inherited deafness and blindness: nil cases amongst PAs
  - Specific mutations within same gene confer different phenotype OR deafness-related CDH23 mutations mostly homozygous or compound heterozygous

Zhang Q et al. Am J Hum Genetics 2017
CABLES1 mutations - a rare cause of inherited Cushing’s disease?

- **CABLES1** located on 18q11.2
  - Cell cycle regulator activated in corticotroph cells in response to glucocorticoids (negative feedback)
- CABLES1 protein expression lost in ~50% ACTH tumours
- 146 sporadic paediatric CD and 35 adult CD
  - 4 (2.2%) potentially pathogenic germline CABLES1 heterozygous missense variants (2 young adults, 2 children)
    - *in vitro* these blocked cell growth in AtT20 cell lines
  - But no LOH of CABLES1 in PA of these patients
  - Tumours all macroadenomas with elevated Ki67 and USP8 negative
  - One parent of each paediatric case asymptomatic carriers of same CABLES1 mutation
    - Suggests low penetrance gene

*Hernandez-Ramirez L et al ERC 2017*
Somatic mutations in classic cancer genes rare in pituitary tumours

- Mutations in classic tumour suppressor genes (e.g. p53) and oncogenes (Ras) rare in pituitary tumours
- Majority of pituitary tumours undergo oncogene-induced senescence
- Chromosomal instability a feature of pituitary tumours: gains and losses
- Pttg1: oestrogen-regulated activating oncogene
  - Frequently over-expressed in pituitary tumours
Mechanisms of pituitary tumourigenesis

- Growth factor and receptor dysregulation
- Cell adhesion and matrix proteins (loss E-cadherin)
- Hormonal stimulation, dysregulated feedback
- Cell cycle gene dysregulation
  - $pRb/p16/cyclin\ D1/CDK4$ pathway altered in 80% PA
- Epigenetic changes esp. promoter hypermethylation and gene inactivation ($Rb, p16, PTAG, GADD45$)
- Pituitary stem cells
- Aberration in signalling pathways (esp. cAMP)
  - 30% GH adenomas mutations in $Gsp$ oncogene
  - 40% ACTH adenomas mutations in $USP8$
- Gain of function – sustained EGF signaling
Genome-wide somatic mutational landscape of PAs

- 125 PAs across 7 PA subtypes
- Low number of somatic mutations per tumour across all 7 PA subtypes (mean 3.3/exome)
  - Confirmed recurrently occurring mutations in GNAS (amongst GH), USP8 (amongst ACTH)
- 32% of PAs though had high CNV alterations (>80% genome) – chromosomal instability
- GH, PRL, plurihormonal and ACTH tumours enriched for somatic mutations in overlapping molecular pathways (Raf/ERK, mTOR/Akt, cAMP)
- 28% of PAs had a mutation in a potentially actionable gene in one of 7 cancer-related pathways

Song ZJ et al Cell Research 2016
Summary

• Pituitary development is a complex process coordinated by a multitude of genes – abnormalities in which lead to various phenotypes

• HH/KS and congenital hypopituitarism form part of a spectrum of pituitary insufficiency disorders

• Increasing genes implicated in pituitary disease being described

• Variable phenotypes and incomplete penetrance of pituitary disease may partly be explained by digenic inheritance or mutational load

• Sporadic pituitary tumours commonly display chromosomal instability without mutations in classic cancer genes
Thank you!

PAST AND CURRENT
HORMONES AND CANCER GROUP
KINGHORN CENTRE FOR CLINICAL GENOMICS

Sunita de Sousa
Mark McCabe
Mark Cowley
Marcel Dinger
Familial hyperprolactinaemia

- Germline loss-of-function PRLR mutation as a cause of familial HyperPRL
- Associate with oligomenorrhoea and infertility but not with PRLomas
- PRL insensitivity
- In PRLR-null mice they get pituitary hyperplasia and large tumours by late adulthood
  possible subtle predisposing factor

\[ \text{c.a635G PRLR mutation} \]

Newey P et al NEJM 2013