Overgrowth Disorders and the POD Study

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What are rare genetic overgrowth disorders?

Large and diverse group of disorders characterised by increased growth

Often associated with intellectual disability and a wide range of health problems

Lifelong conditions that usually present in early childhood
Overgrowth

**Generalised** overgrowth:
Tall stature and/or macrocephaly
> 2 SD (>98\textsuperscript{th} centile)

**Lateralised** overgrowth:
One side of the body larger than the other
(hemihypertrophy/hemihyperplasia)

**Regional** overgrowth:
Increase in size of a region(s) of the body

From Mirzaa et al. GeneReviews 2013
Associated features

Development, learning and behaviour
- Developmental delay, intellectual disability, autism spectrum disorder

Congenital anomalies
- Cardiac, renal, abdominal wall etc.

Other medical problems
- Neonatal hypoglycaemia, hypotonia, scoliosis, seizures, etc.

Increased risk of tumours
- Childhood embryonal tumours e.g. Wilms
- Adult onset tumours
Example of a well known overgrowth disorder: Sotos syndrome

- Tall stature and macrocephaly
- Facial features
  - Advanced bone age
- Intellectual disability
- Behavioural problems
- Seizures
- Congenital heart disease
- Neonatal jaundice
- Hypotonia
- Feeding difficulties
- Congenital renal anomalies
- CNS abnormalities
Example of a well known overgrowth disorder: **PTEN hamartoma tumour syndrome**

Bannayan-Riley-Ruvalcaba syndrome (BRRS) in childhood:
- Macrocephaly
- Developmental delay
- Intestinal polyps
- Lipomas
- Genital freckling

Cowden syndrome in adulthood:
- Macrocephaly
- High cancer risk in adulthood (breast 85%, thyroid 35%, endometrial 28% renal 35%): cancer surveillance program recommended
- Skin lesions (e.g. trichilemmomas, papillomatous papules)
Clinical diagnostic challenges

Patients with non-specific clinical features

Patients with clinical features that do not fit any known disorder

Different disorders can have significant phenotypic overlap

A single disorder can present with diverse phenotypes
Advances in DNA Sequencing

1980’s
Radioactive sequencing

1995
Fluorescent sequencing

2003
Capillary based Fluorescent sequencing

2010
Next Generation sequencing
Epigenetic regulation genes

e.g. **DNMT3A** – encodes a DNA methyltransferase

e.g. **CHD8** - encodes a chromatin remodelling enzyme (helicase)

e.g. **NSD1** and **EZH2** encode histone methyltransferases

From Rajender, Avery & Agarwal *Mutation Research* 2011
PI3K/AKT pathway genes

PIK3CA, MTOR, AKT3, PTEN, PPP2R5D

Despite a rapid increase in the rate of discovery of overgrowth genes:

The full phenotypes of these emerging disorders are not yet known.

Information about likely prognosis, complications, need for investigations, utility of screening for tumours etc. is not available.

Diagnostic molecular testing is not readily available for many newly discovered genes.

-> Need to translate research findings into clinical benefit.
Phenotyping of Overgrowth Disorders (POD) study

The POD study aims to develop a cohort of individuals with rare genetic overgrowth disorders and perform deep phenotyping on this cohort.

**Phenotype**: deviation from normal morphology, physiology, or behaviour

**Deep phenotyping**: the complete and detailed understanding of phenotypic abnormalities associated with each disease entity
- ascribe pathogenicity to genomic variants
- establish genotype-phenotype correlations
- stratify patients into subpopulations and enable precision medicine

Peter Robinson. Deep Phenotyping for Precision Medicine. Human Mutation 2012
POD study recruitment

Inclusion criteria
Height and/or head circumference >2 SD with an associated feature (e.g. developmental delay) or

Height and/or head circumference >3 SD or

Lateralised or regional overgrowth

Participants
Ideally trios (proband, mother, father)

Samples for molecular genetic analysis
Ideally blood (saliva acceptable)
If regional overgrowth: affected tissue i.e. skin biopsy
Phenotyping data

Demographic, perinatal, growth, medical, developmental, family history, dysmorphology and genetic investigations

OpenClinica electronic data capture system to establish a clean data set

Human Phenotype Ontology (HPO) for coding data - accurate and reproducible
Molecular genetic investigations in the POD study

Custom targeted NGS panel of 44 genes associated with a known human overgrowth phenotype

| AKT1  | HERC1  | PTCH1 |
| AKT2  | HIST1H1E | PTEN  |
| AKT3  | KPTN   | RNF125 |
| APC2  | MED12  | RNF135 |
| BRWD3 | MTOR   | SETD2  |
| CCND2 | NFIA   | SUFU   |
| CDKN1C| NFIX   | SUZ12  |
| CHD4  | NLGN2  | TBC1D7 |
| CHD8  | NPR2   | TCF20  |
| DICER1| NSD1   | ZBTB20 |
| DIS3L2| PDGFRB |        |
| DNMT3A| PIGA   |        |
| EED   | PIK3CA |        |
| EZH2  | PIK3R2 |        |
| FGFR3 | PPP2R5B|        |
| FIBP  | PPP2R5C|        |
| GPC3  | PPP2R5D|        |

Whole exome sequencing performed on selected panel-negative cases
Eligible patients without a diagnosis offered entry to the 100K Genomes Project
NGS panel methods

Library preparation: Agilent SureSelect QXT Target Enrichment

Sequencing: Illumina MiSeq

Bioinformatic analysis: Agilent SureCall software

Variant classification: ACMG Standards and Guidelines

Confirmation of pathogenic variants: Sanger sequencing in the service lab

Participant 002.0

Born at term BW 3.6 kg (+0.4 SD) to unrelated parents

Age 12 height +1.3 SD, weight +0.8 SD, OFC +2 SD

Global developmental delay, hypotonia, moderate-severe ID

Anxiety and social interaction difficulties

Bilateral strabismus and reduced visual acuity, conductive hearing loss and ingrowing toenails

Investigations: Karyotype, CGH microarray, *NSD1*

Overgrowth panel: pathogenic variant in *NFIX*
Malan syndrome

Malan et al. 2010

Tall stature
Macrocephaly
Intellectual disability
Autism spectrum disorder
Characteristic facial features
Skeletal anomalies

Impact of diagnosis on patient care:
Emerging evidence of aortic dilatation
Echocardiogram requested

Malan et al. Distinct effects of allelic NFIX mutations on nonsense-mediated mRNA decay engender either a Sotos-like or a Marshall-Smith syndrome. Am J Hum Gen 2010
Participant 030.0

Born at 40+12 to unrelated parents BW 4.7kg (+1.4 SD), OFC +2.6 SD

Age 7 height +0.8 SD, weight +1.0 SD, OFC +3.4 SD

Developmental delay, sensory processing disorder, attention deficit disorder

Neonatal hypoglycaemia, subsequently well

Investigations: MRI brain, CGH microarray, \textit{PTEN}

Overgrowth panel: pathogenic variant in \textit{PPP2R5D}
PPP2R5D-associated neurodevelopmental disorder

DDD Study 2015, Loveday et al. 2015, Houge et al. 2015, Shang et al. 2016

Macrocephaly
Hypotonia
Developmental delay
Intellectual disability (milder in individuals with E200K)

Impact of diagnosis on patient care:
7 years of neurosurgical follow up discontinued
Evidence for additional support at school and proactive management of ADD

Panel testing results to date

7 pathogenic variants / 50 participants tested: diagnostic rate – 14%

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Gene</th>
<th>Variant</th>
<th>Diagnosis</th>
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<tbody>
<tr>
<td>Tall stature +5 SD, weight +3.7 SD, ID, autistic traits</td>
<td>DNMT3A</td>
<td>Nonsense c.499C&gt;T</td>
<td>Tatton-Brown-Rahman syndrome</td>
</tr>
<tr>
<td>Macrocephaly +2 SD, ID, hypotonia, anxiety, squint, conductive hearing loss</td>
<td>NFIX</td>
<td>Missense c.248T&gt;G</td>
<td>Malan syndrome</td>
</tr>
<tr>
<td>Regional overgrowth, cutis marmorata</td>
<td>PIK3CA</td>
<td>Mosaic missense c.2740G&gt;A</td>
<td>PIK3CA-related overgrowth (PROS)</td>
</tr>
<tr>
<td>Tall stature &gt;3 SD, dev delay, autistic traits, anal stenosis</td>
<td>CHD8</td>
<td>Frameshift c.716delA</td>
<td>CHD8-associated neurodevelopmental disorder</td>
</tr>
<tr>
<td>Macrocephaly +3.4 SD, dev delay, ADHD</td>
<td>PPP2R5D</td>
<td>Missense c.502G&gt;A</td>
<td>PPP2R5D-associated neurodevelopmental disorder</td>
</tr>
<tr>
<td>Tall stature +3SD, dev delay, craniosynostosis, cystic dysplastic kidney</td>
<td>NSD1</td>
<td>Missense c.5791T&gt;C</td>
<td>Sotos syndrome</td>
</tr>
<tr>
<td>Macrocephaly +2 SD, ID, autistic traits, ADHD</td>
<td>PTCH1</td>
<td>Frameshift c.2611-2624del</td>
<td>Gorlin syndrome</td>
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62 probands recruited (plus parent participants)

Approved for recruitment at 14 centres across the UK through the Musketeer’s Memorandum (NIHR UK Rare Genetic Disease Research Consortium Agreement)

Panel testing, whole exome sequencing and recruitment to 100K Genomes ongoing

Study end date February 2019
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