The Expert's view on growth hormone disorders
Disclosure

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Declared receipt of grants and contracts from Ipsen; receipt of honoraria or consultation fees from Teva Pharmaceuticals, EMD Serono, Versartis, Pfizer and OPKO Biologics; to be Member of a company advisory board, board of directors or other simila groups of OPKO Biologics; to be stakeholder in OPKO Biologics.
Genomic insights into growth and its disorders

Andrew Dauber
USA
Case Presentation

• 20 year old male
• Birth weight -3.7 SDS
• Birth length -6.8 SDS
• Adult height 146.6 cm (~4’10”, -4.17 SDS)
• Large head (+2.4 SDS)
• Mother 5’8”, Father 6’5”
• Normal intelligence
• Normal IGF-1, IGFBP-3, and growth hormone levels on stimulation testing

What actually makes you grow?

- GH/IGF-1 axis
- Chondrocyte proliferation and differentiation
- Growth plate matrix and signaling (Proteoglycans, Hedgehog, TGF, etc...)
- DNA replication
- Cell division (Cell cycle, centrosomes, microtubules, etc...)
- Nutrient and energy regulation
- And many more...
HOW CAN WE USE GENOMIC TECHNOLOGIES TO UNDERSTAND THE BIOLOGY OF GROWTH?
Allele Frequency

Effect Size

Sequencing

Rare variants causing monogenic disorders

Very Hard
(lots of sequencing)

Low frequency variants with moderate effects

GWAS

Common variants contributing to complex diseases/traits

Allele Frequency
What is GWAS?

A Creation of microarray
- Defined oligonucleotides
- Photolithography or other technique
- DNA "chip"
- Sample DNA
- Hybridization
- Fluorescent label

B Differential hybridization
- SNP1
- SNP2
- Person 1
- Person 2
- Person 3
- G-C → T-A
- A-T → G-C

C Detection
- Patient 1
- Patient 2
- Patient 3
- Patient 4

D Interpretation
- SNP1
- SNP2
- SNP3
- SNP4
- SNP5
- SNP6
- SNP7
- SNP8
- SNP9
- SNP10
- SNP11
- SNP12
- SNP13
- SNP14
- SNP15
- SNP16
- SNP17
- SNP18
- SNP19
- SNP20
- SNP21
- SNP22
Common Variants and Height

Height is highly heritable ($h^2 \sim 0.8$)

N = 253,288

697 variants at genome-wide significance

423 loci

20% of height heritability

Finding Biological Pathways of Growth

Same as previous:
Collagen/extracellular matrix
IGF/GH signaling
TGF-beta signaling
BMP/Noggin
Hedgehog signaling
Chromatin

New:
FGF signaling
WNT signaling
Osteoglycin
TWIST/RUNX2
NPR2/NPPC
Bone/cartilage development

Many loci still have no known connection to biology of human growth

Slide adapted from Joel Hirschhorn
HOW CAN WE USE GENOMIC TECHNOLOGIES TO UNDERSTAND AN INDIVIDUAL PATIENT’S SHORT STATURE?

- Copy number analysis
- Exome sequencing
Copy Number Variation
Duplications
Deletions
CNVs in Short Stature

Rare Copy Number Variants Are a Common Cause of Short Stature

Diana Zahnleiter¹, Steffen Uebe¹, Arif B. Ekici¹, Juliane Hoyer¹, Antje Wiesener¹, Dagmar Wieczorek², Erdmute Kunstmann³, André Reis¹, Helmuth-Guenther Doerr⁴, Anita Rauch⁵, Christian T. Thiel¹*


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ARTICLE

Copy number variants in patients with short stature

Hermine A van Duyvenvoorde⁴*,¹,²,³, Julian C Lui⁴, Sarina G Kant³, Wilma Oostdijk¹, Antoinet CJ Gijsbers³, Mariëtte JV Hoffer³, Marcel Karperien⁵, Marie JE Walenkamp⁶, Cees Noordam⁷, Paul G Voorhoeve⁸, Verónica Mericq⁹, Alberto M Pereira², Hedi L Claahsen-van de Grinten⁷, Sandy A van Gool¹, Martijn H Breuning³, Monique Losekoot³, Jeffrey Baron⁴, Claudia AL Ruivenkamp³ and Jan M Wit¹
HOW CAN WE USE GENOMIC TECHNOLOGIES TO UNDERSTAND AN INDIVIDUAL PATIENT’S SHORT STATURE?

• Copy number analysis
• Exome sequencing
Exome Sequencing
Approach to Variant Filtering

All variants identified from sequencing

Variants that pass QC

Technical Validation

Rare Variants

Population based allele frequency

Variant effect on protein

In Silico Functional Prediction

In Vitro Functional Effect

Known Pathogenic Gene

CNV of Gene with Same Phenotype

Segregate with phenotype

Pedigree Analysis

Non-synonymous Variants

Animal Models
Back to our case

- 20 year old male
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Genomic Evaluation

- Over 20 year period, seen by 3 senior endocrinologists and 2 geneticists
- X-rays sent to International Skeletal Dysplasia Registry
- Diagnosed as “Unknown Syndrome”

<table>
<thead>
<tr>
<th>Genetic Test</th>
<th>Cost</th>
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<tbody>
<tr>
<td>Karyotype</td>
<td>$315</td>
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<tr>
<td>Chromosomal Microarray</td>
<td>$1595</td>
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<tr>
<td>PTPN11 Gene Sequencing (Noonan Syndrome)</td>
<td>$1400</td>
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<tr>
<td>SOS1 Gene Sequencing (Noonan Syndrome)</td>
<td>$2400</td>
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<tr>
<td>FGFR3 Mutational Analysis (Achondroplasia/Hypochondroplasia)</td>
<td>$310</td>
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<tr>
<td>SHOX Gene Sequencing</td>
<td>$990</td>
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<tr>
<td>Russell-Silver Testing (H19 Methylation and UPD of Chromosome 7)</td>
<td>$900</td>
</tr>
<tr>
<td>Total Cost of Genetic Testing Performed</td>
<td>$7910</td>
</tr>
<tr>
<td>Clinical Exome Sequencing</td>
<td>$7000 - 7900</td>
</tr>
</tbody>
</table>

- Exome sequencing revealed novel homozygous frameshift mutation in *CUL7* – a causative gene for 3-M syndrome.
- This syndrome has been described in ~100 people in the world.
- Re-examination noted small testicular volume with implications for fertility.

Familial Short Stature with Advanced Bone Age

Affected individuals

- Short stature (-1.9 to -4.2 SDS)
- Advanced BA (1.5-4.0 y over CA) and/or early growth cessation (age 10-13 y) despite normal puberty
- Negative endocrine evaluations
- In family 3: Early onset osteoarthritis with osteochondritis dissecans
- Other features:
  - Midface hypoplasia
  - Brachydactyly
  - Exaggerated lumbar lordosis
Family 1: c.272delA
Family 2: c.2026+1G>A
Family 3: c.7064T>C

Gene structure

Family 1: Frameshift p.Arg93Alafs*41
Family 2: Splice site c.2026+1G>A
Family 3: Missense p.L2355P

Protein structure

ACAN Haploinsufficiency: The story continues

102 mutation positive individuals from 20 families
Not all patients have severely advanced bone age.
Growth Failure with Elevated IGF-1

M1
39 yr
159.2 cm
-0.64 SD

D1
46 yr
174 cm
-0.40 SD

S1
22 yr
149.3 cm
-2.16 SD

S2
20 yr
153.9 cm
-1.45 SD

P1
18 yr
138.3 cm
-3.82 SD

P2
13 yr
137.2 cm
-2.68 SD

P3
9 yr
118.2 cm
-2.72 SD
Biochemical Parameters

<table>
<thead>
<tr>
<th>Age</th>
<th>IGF-1</th>
<th>IGFBP-3</th>
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</thead>
<tbody>
<tr>
<td>6y10m</td>
<td>517.3</td>
<td></td>
</tr>
<tr>
<td>7y5m</td>
<td>534.3</td>
<td></td>
</tr>
<tr>
<td>7y7m</td>
<td>545.6</td>
<td>6.4</td>
</tr>
<tr>
<td>8y6m</td>
<td>726.6</td>
<td></td>
</tr>
<tr>
<td>9y0m</td>
<td>698.3</td>
<td></td>
</tr>
<tr>
<td>10y10m</td>
<td>519</td>
<td>7.3</td>
</tr>
<tr>
<td>11y5m</td>
<td>564</td>
<td></td>
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<tr>
<td>11y10m</td>
<td>786</td>
<td></td>
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<tr>
<td>12y11m</td>
<td>702</td>
<td>6.4</td>
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<tr>
<td>13y2m</td>
<td>825</td>
<td>3.7</td>
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<tr>
<td>14y0m</td>
<td>895</td>
<td>8.7</td>
</tr>
<tr>
<td>15y8m</td>
<td>1031</td>
<td>8.1</td>
</tr>
<tr>
<td>18y</td>
<td>1060</td>
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</table>

Peak GH >50 ng/ml on stim testing
- 12y6m
  - IGF-1 657
  - BP3 4.4
  - Peak GH 14.7

- 13y7m
  - IGF-1 935
<table>
<thead>
<tr>
<th>Age</th>
<th>IGF-1</th>
<th>BP3</th>
<th>Peak GH</th>
</tr>
</thead>
<tbody>
<tr>
<td>8y4m</td>
<td>636</td>
<td>4.5</td>
<td>37</td>
</tr>
<tr>
<td>9y6m</td>
<td>831</td>
<td></td>
<td></td>
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</table>
Homozygosity Mapping

- SNP array performed on 3 affected siblings
- Four regions of overlapping homozygosity

<table>
<thead>
<tr>
<th>Chr</th>
<th>Start</th>
<th>End</th>
<th>Length (MB)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>171755170</td>
<td>178019772</td>
<td>6.3</td>
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<tr>
<td>3</td>
<td>186205930</td>
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<td>8.5</td>
</tr>
<tr>
<td>8</td>
<td>54308719</td>
<td>61809918</td>
<td>7.5</td>
</tr>
<tr>
<td>13</td>
<td>74096953</td>
<td>84845106</td>
<td>10.7</td>
</tr>
</tbody>
</table>

- Performed exome sequencing on 1 proband
All variants identified from sequencing
87,599

Variants that pass QC

Rare Variants
583

Variant effect on protein

In Silico Functional Prediction

In Vitro Functional Effect

Known Pathogenic Gene

CNV of Gene with Same Phenotype

Animal Models

Technical Validation

Population based allele frequency

Segregate with phenotype

Pedigree Analysis

Non-synonymous Variants
119

19 homozygous

2 in shared block of homozygosity

1 segregated in unaffected sisters
What is **PAPPA2**?

- Cleaves IGF Binding Proteins 3 + 5
- Regulates release of free IGF-1
- Mouse model with post-natal growth retardation
Knock Out Mouse

Q: How do you prove a new gene?
A: Sharing information with collaborators is key to success.

Jesus Argente had already identified a second family!

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Figure 1

Courtesy of Ansh labs
• Mentors
  – Joel Hirschhorn
  – Ron Rosenfeld
• Cincinnati Team
  – Vivian Hwa
  – Melissa Andrew
  – Shayne Andrew
  – Leah Tyzinski
  – Priya Kumar
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  – Claus Oxvig
  – Jan Frystyk
  – Vardhini Desikan
  – Horacio Domene
  – Radhika Muzumdar
  – Shoshana Yakar
The Cincinnati Center for Growth Disorders

➤ Clinical Referrals

➤ Research Collaborations
  – Genomics
  – Molecular Analysis of Unsolved Cases
  – Translational Biology
  – Expertise in the GH/IGF-1 axis
  – Clinical Trials

Contact: GROWTH@CCHMC.ORG

Andrew Dauber, MD, MMSc, Program Director & Director of Clinical Research
Philippe Backeljauw, MD, Clinical Director
Vivian Hwa, PhD, Basic Research Director

*External Advisory Board:* Joel Hirschhorn, MD; Ron G Rosenfeld, MD