International symposium on IGF-1, GH and Ghrelin/GHS

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Genotype:phenotype Analysis

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The concept of genotype:phenotype relationships in endocrinology evolved from evidence that a range of genetic defects can influence phenotypic expression based on the degree of disturbance of key endocrine mechanisms.

For example in congenital adrenal hyperplasia certain $CYP21$ mutations cause different degrees of steroidogenic disruption resulting in a range of clinical phenotypes.
What is the evidence that genotypes determine phenotypes?
- Across the continuum of primary IGF-I deficiency disorders
- Within genotypes of certain key candidate genes

Genotype:phenotype relationships in Primary IGF-I deficiency disorders

- >300 cases (~60 mutations) (GHBP–)
  - 9 cases (GHBP+)
  - 10 cases (GHBP+)
  - 6 cases
  - 20 cases
  - 14 cases

'Extreme and mild phenotypes'
'Severe and mild phenotypes'
Role of GH and IGF-I in Prenatal/Postnatal Growth

Data derived from murine models and limited case reports
Birth weights in Patients with *IGF1* and *IGF1R* defects

![Box plot showing birth weights in patients with IGF1 and IGF1R defects.](image)

- **n= 6** for IGF1
- **n= 14** for IGF1R

*Birthweight (g)*

*Mutation*
Role of GH and IGF-I in Prenatal/Postnatal Growth

Data derived from murine models and limited case reports
Height SDS in patients with ALS, GH receptor and STAT5b mutations

Dr Alessia David – PhD Thesis 2009
Relationships between GH-IGF-I axis defects and height

David A et al. 2011
Continuum of phenotypes in primary IGF-I deficiency

Homozygous $GHR$ mutations

An out-dated view

‘Severe Primary IGF Deficiency always has the phenotype of extreme GH resistance’

Age 7.4 yrs Height -8.2 SD
Severe Primary IGF-I Deficiency
GH insensitivity syndrome (GHIS)

Homozygous \textit{GHR} mutations

‘A characteristic facial appearance’
Idiopathic short stature

GH secretion abnormalities

Extreme GH deficiency

Idiopathic short stature

GH resistance

Primary IGF Deficiency

Extreme GH resistance

GH-IGF-I axis continuum

Height SDS and IGF-I SDS across the continuum of GH-IGF-I axis defects

Savage MO et al. Clin Endocrinol 2010, 72; 721-728
In 2011, the skeptics still believe;

‘… a continuum of GH sensitivity…no substantive data to support this’

‘No population data to support the concept of a continuum’

‘Concept of continuum …totally lacking in evidence. This is pure invention’
Four series of patients with Primary IGFD to be examined for evidence of a continuum of phenotypic and biochemical abnormalities

2. GHIS subjects treated with IGF-I/IGFBP-3 complex – Camacho-Hübner et al. ENDO 2007
3. Subjects with Primary IGFD investigated at IGFD Research Center, Oregon - Rosenfeld & Hwa Horm Res 2009
4. Subjects with Primary IGFD studied at St Bartholomew’s Hospital, London – A David et al. 2011
Heights of Patients with GH Insensitivity Syndrome from the European Series (N=82)

Woods et al JCEM 1997

IGF-I generation: IGF-I increase <15 ng/ml, IGFBP-3 increase <0.4 mg/L
Height SDS in patients with GHIS (N=36) treated with the rhIGF-I/rhIGFBP-3 complex

Camacho-Hübner C et al. 2007
Serum IGF-I and height SDS values in patients with Primary IGFD (Ht <-3 SD, IGF-I <-3 SD) investigated in the IGFD Research Center at OHSU, Portland, Oregon

Shown with kind permission of Vivian Hwa and Ron Rosenfeld
Evidence for a ‘Continuum’ of GH-IGF-I axis defects

Dr Alessia David PhD Thesis 2009

Children with short stature and GH resistance/Primary IGF-I Deficiency (N=70)

Idiopathic short stature

Primary IGF Def

Extreme GH resistance

Height SDS

0

-10

-8

-6

-4

-2

0
Having established a continuum of phenotypic and biochemical data in patients with Primary IGF deficiency…

… does genotype determine phenotype within this continuum?
Phenotypic variation correlates with the biological defect (IGF-I) in patients with GHR mutations.

Ecuador GHIS Patients
E180 splice GHR mutation

Serum IGF-I (µg/L) vs. SDS for Height

P<0.01

Thanks to Dr Arlan Rosenbloom

Homozygous or compound heterozygous GH receptor mutations causing GH resistance (n>60)

Exon

Signal peptide

Extracellular domain
GH binding

Transmembrane domain

Intracellular domain
GH signaling

Signal peptide

GHBP normal or increased

GHBP undetectable

GHBP levels in 58 patients with growth hormone insensitivity investigated at St Bartholomew’s Hospital, London

Dr Alessia David Endocrine Rev 2011
Dominant negative heterozygous GHR mutations cause a mild Primary IGF deficiency phenotype

Single heterozygous mutation (G to C) at the acceptor splice site of exon 9

Normal facial features
Height SDS -3.6
IGF-I = -2.4 SDS

Single heterozygous mutation (G to A) at the donor splice site of exon 9

Prominent forehead and saddle nose
Height SDS -3.0 to -3.5

Ayling et al Nat Genet 1997

Iida et al JCEM 1998
Dominant Negative Heterozygous GHR Mutation Presenting with Growth Failure

A dominant-negative mutation of the growth hormone receptor causes familial short stature


Glucagon test:

<table>
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<th>TIME</th>
<th>GLUCOSE MMOL/L</th>
<th>H GROWTH HORMONE MU/L</th>
<th>CORTISOL nmol/L</th>
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<td>180</td>
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<td>157</td>
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Thanks to Dr Charles Buchanan, King’s College Hospital, London
GH receptor pseudoexon mutation presenting as idiopathic short stature

An Intronic Growth Hormone Receptor Mutation Causing Activation of a Pseudoexon Is Associated with a Broad Spectrum of Growth Hormone Insensitivity Phenotypes


Centre for Endocrinology (A.D., C.C.H., F.M.M., S.A.A., A.J.L.C., M.O.S., L.A.M.), William Harvey Research Institute, Bart’s and the London, Queen Mary, University of London, London EC1M 6BQ, United Kingdom; Pediatric Endocrinology Division (A.B., S.T.), Infant’s and Children’s Hospital of Brooklyn at Maimonides, Brooklyn, New York 11219; Department of Paediatrics (S.J.R.), Heartlands Hospital, Birmingham B9 5SS, United Kingdom; Department of Paediatrics (G.E.B.), Leeds General Infirmary, Leeds LS1 3EX, United Kingdom; and Endocrine Science Research Group (P.E.C.), School of Medicine, University of Manchester, Manchester M13 9PT, United Kingdom

Patients of Dr Svetlana Ten, courtesy of Dr Amrit Bhangoo, Maimonides Hospital, Brooklyn, NY
Possible pathogenetic mechanism for differing phenotypes in GHR pseudoexon mutation patients

The different clinical phenotypes may be due a variation in the ratio of normal to mutant protein.
Height SDS with sub-division of GHR, STAT5b and ALS defects according to type of mutation

Dr Alessia David et al. 2011
IGF-I Levels According to GHR Mutation Type (n=41)

- MISSENSE
- FRAMESHIFT
- STOP
- PSEUDOEXON

p<0.01

A David et al. 2011
The Phenotype-genotype algorithm

Child with height < -2 SD

History enquiry for consanguinity, family history of short stature, motor, intellectual milestones, hypoglycemia, birth weight/length, deafness, immune deficiency

Low birth weight/length, intellectual delay, deafness

- Microcephaly, facial dysmorphism
  - IGF-I deficiency, normal IGFBP-3, ALS, increased GH, insulin excess
    - IGF-I mutation
    - IGF-I mutation: bioinactive IGF-I
  - Normal or increased IGF-I, normal IGFBP-3
    - IGFR mutation

Normal birth weight/length

- Physical examination for auxology assessment, facial dysmorphism, immune deficiency
  - Severe growth failure
    - Immune deficiency
  - Mild growth failure
    - Immune deficiency

- Noonan syndrome phenotype
  - PTPN11 mutation

IGF-I, IGFBP-3, ALS, deficiency, increased GH, decreased or normal GHBP
  - GHR mutation
  - IGFALS mutation

IGF-I, IGFBP-3, ALS deficiency, insulin excess

GH deficiency, IGF-I deficiency, anti-GH antibodies
  - GH1 deletion type 1A IGHD
  - GH1 mutation, bioinactive GH
  - STAT5b mutation

Normal GH, IGF-I deficiency, GH response in IGFGT
  - Normal GH, IGFBP-3, ALS deficiency, normal GHBP
  - IGF-I, IGFBP-3, ALS, deficiency, increased GH, decreased or normal GHBP
  - IGF-I, IGFBP-3, ALS deficiency, insulin excess
Genotype:phenotype Analysis

Final thoughts

• A continuum of genetic and phenotypic defects exists across the spectrum of primary IGF deficiency disorders.

• Defects in particular genes influence phenotypes in fetal and/or postnatal growth and associated features such as immune deficiency.

• GHR defects, considered to be the hallmark of GHIS as in the extreme GHIS phenotype, are heterogeneous. Genotypes associated with mild phenotypes have been identified.

• The strongest relationship with phenotype is not the original genotype, but biological variables, such as IGF-I, which are the closest indices of the degree of endocrine dysfunction.
Genotype-phenotype

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    • Dina Ramadan
Thank you for your attention!