Genetics vs. Epigenetics in Pediatrics

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Evolution vs. Development =

The Evo-Devo Field

Phylogeny vs. Ontogeny

Genetics vs. Epigenetics
Genetics vs. Epigenetics

- Complex Systems
- Evolutionary and Developmental Stressors
- Stress and Evo-Devo
“Πολλά τα δεινά κ’ουδέν ανθρώπου δεινότερον πέλλει….”

“There are many wonderful things and nothing is more wonderful than the human...”

Σοφοκλής/ Sophocles
496-406 BCE
HUMAN COMPLEXITY: POST(EPI)GENOMIC ERA

Human genome:
- About 3+3 billion bases, 98% formerly “junk DNA”
- About 20 thousand protein-coding genes
- About 24 thousand ncRNA-coding genes
- About 16 thousand pseudogenes
- About 100-140 thousand transcripts
  (mRNAs, ncRNAs = miRNAs, lncRNAs, piRNAs, cRNAs, eRNAs)
- About 200-260 thousand proteins

Single nucleotide polymorphisms (snp’s or snv’s), microsatellites or copy number variants:
- >25 million snp’s (snv’s), 1.5 million indels
- About 20 million microsatellites
- >5000 cnv’s (many million bases) 91.1 vs. 0.9%

Over 50 k disease-related mutations
- 60% of promoters have CpG islands, >1 million reg. regions

↓

EPIGENETICS (Epi)mutations
HUMAN COMPLEXITY: SOME HUMAN BRAIN NUMBERS

~ 100 billion neurons \( (100 \times 10^{12}) \times >10,000 \)
synapses per neuron = \( >10^{18} \) synapses, peak at 2 ys

~ 100,000 km of fibers

~ 1 trillion or more glial cells

~ 1.25 terabytes

~ 15 Watt lamp (2% of BW uses 20% energy)

Plasticity
Man or mouse? Both the presence and absence of transcription factor binding sites in a genome, as well as the binding of transcription factors to sites that are present, can differ between species and may account for differences in gene expression and phenotype.
• About 250 genes “HARs”

- Mostly regulatory noncoding areas and transcription factors
The diagram illustrates the exponential growth of power density over time, with different stages marked by different stages of development:

- **Hunter-gatherers**
- **Agriculturists**
- **Industrialists**
- **Society**
- **Brains**
- **Animals**
- **Plants**
- **Planets**
- **Stars**
- **Galaxies**

The vertical axis represents power density (W/kg), while the horizontal axis represents time (years), with a logarithmic scale. The graph shows a significant increase in power density over time, particularly in the recent past. The diagram is from Chaisson E, New Scientist 2009.
Man and his/her civilization are of unique complexity in the known universe.

Complex systems are in a dynamic disequilibrium that requires energy to be sustained.

Complex systems have organizing principles and follow mathematic rules.

SYSTEMS BIOLOGY, SYSTEMS MEDICINE NARRATIVE AND PRECISION MEDICINE
Disturbing Forces

Harmony Equilibrium Balance

Counteracting Reestablishing Forces

Stressors (Physical, Emotional)

Homeostasis

Pythagoras = Harmony
Alcmaeon = Iso-nomia
Walter Cannon = Homeo-stasis
Stress is the State of Threatened (or Perceived Threatened) Homeostasis
Genetics vs. Epigenetics

- Complex Systems
- Evolutionary and Developmental Stressors
- Stress and Evo-Devo
Evolutionary and Developmental Stressors

- Starvation
- Dehydration/hemorrhage (gastroenteritis, trauma)
- Injurious agents (infections, toxic substances)
- Adversaries (anticipation, minimization of exposure)
- Tissue injury
- Social bonding disruption
Raphael, 16th Century CE
Preformation (Plato)

(unfolding of preformed tissues = Genesis)

Epigenesis (Aristotle)

(environmental influences on tissues = Epi-genesis)
Jean-Baptiste Lamarck (1744–1829)
Charles Robert Darwin (12 February 1809 – 19 April 1882)
Modern definitions

“Epigenetics are the causal interactions between genes and their products, which bring the phenotype into being” 1942

Conrad H Waddington 1900-1975
The Strategy of Genes, MacMillan 1957
“The Epigenetic Landscape”
Modern definitions

“Genetics proposes; Epigenetics disposes”

Medawar and Medawar 1983
Genetics vs. Epigenetics vs. Phenotype

Piano vs. Pianist vs. Music
ENVIRONMENT

GENETICS

Genetic variants

Stochastic epigenetic variants

Tissue differentiation

EPIGENETICS

Metastable epigenetic variants

Heritable epigenetic variants

Environment-derived epigenetic variants

Lifestyle, etc.
<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>4th c. BCE</td>
<td>Aristotle: <strong>Epigenesis, Embryology, Phylogeny vs. Ontogeny</strong></td>
</tr>
<tr>
<td>1801</td>
<td>Jan-Baptiste Lamarck: <strong>Role of use and disuse in heritability, inheritance of acquired properties</strong></td>
</tr>
<tr>
<td>1859</td>
<td>Charles Darwin: <strong>Evolution</strong></td>
</tr>
<tr>
<td>1942</td>
<td>Conrad Waddington: <strong>Epigenetics</strong></td>
</tr>
<tr>
<td>1948</td>
<td>Rollin Hotchkiss: <strong>DNA methylation</strong></td>
</tr>
<tr>
<td>1953</td>
<td>Watson, Crick, Franklin, Wilkins: <strong>DNA structure</strong></td>
</tr>
<tr>
<td>1962</td>
<td>John Gurdon: <strong>Differentiation reversal in the frog</strong></td>
</tr>
<tr>
<td>1978</td>
<td>Robert Tijan: <strong>Transcription factors</strong></td>
</tr>
<tr>
<td>90's</td>
<td>David Allis: <strong>Histone acetylation</strong></td>
</tr>
<tr>
<td>90's</td>
<td>Marianne Frommer and Suzan Clark: <strong>DNA methylation of repetitive elements, CpG islands</strong></td>
</tr>
<tr>
<td>1998</td>
<td>Craig Mello and Andrew Fire: <strong>miRNAs</strong></td>
</tr>
</tbody>
</table>
Cytosine

5-Methylcytosine

DNMT1
DNMT3A
DNMT3B
Acetyl-lysine

Lysine
Forms of Inheritance

- Genetic (blueprint)
- Structural
- Steady state
- Epigenetic
- Behavioral/Symbolic (memes)
Components of Epigenetic Processes

- Covalent bonds on DNA
- Post-translational modification of chromatin proteins
- DNA-binding proteins or complexes
- nc RNAs: miRNAs, IncRNAs, piwiRNAs, cRNAs, eRNAs
- Super-enhancers
Epigenetic Mechanisms

- DNA Methylation/demethylation, acetylation/deacetylation
- Covalent histone modifications (Methylation, acetylation, phosphorylation, polyADP-ribosylation)
- Methyl-CpG domain-binding proteins
- Chromatin compacting or unwinding complexes (polycomb-, trithorax groups+)
- Multiple coordinated gene regulators (Cell differentiation)
Profiling Epitranscriptomics

• *Writers or highlighters*

• *Erasers*

• *Readers or decoders*

• *Facilitators (HMG1 and HMG2)*
Gene Activation

Me
K4
Me
K9
Me
K27
Me
K36
Me
K20

CpG Me

Gene Silencing
Gene Activation
Epigenetic Functions

- Embryonic cell differentiation, organ morphogenesis*
- Genomic imprinting*
- X-chromosome inactivation
- Retrotransposon repression
- Somatic stem cell differentiation*
- Immune cell differentiation/ function
- Body composition*
- Labor and delivery
- Puberty
- Sexual orientation
- Right/left handedness
- Stress-related behaviors/somatic changes*
- Memory
Parental Genetic Imprinting

Source of chromosome 11

Both copies from mother: Mouse smaller than normal + problems
Russel-Silver s. vs. Angelman s.

One copy from each parent: Normal mouse
Meg1/Grb10

Both copies from father: Mouse larger than normal + problems
Beckwith-Wiedemann s. vs. Prader-Willi s.
Growth retardation
failure to thrive

Birk-Barel MR
≠ 8q24.3
Mental retardation, Feeding difficulties

Birk-Barel MR
≠ 8q24.3
Mental retardation, Feeding difficulties

Kagami-Ogata s. (upd(14)pat)
≠ 14q32
Parathormone resistance

Overlapping Features
- Aberrant growth
- Asymmetry
- Hypo/hyperglycemia
- Mental retardation

Common Molecular Findings
- (Epi)mutations
- MultiLocus Methylation Defects

General Findings in ID
- Non-Mendelian inheritance
- Environmental contribution

Transient Neonatal Diabetes Mellitus
≠ 6q24
Transient neonatal diabetes

Prader-Willi syndrome
≠ 15q11q13
Growth retardation mental retardation

Angelman syndrome
≠ 15q11q13
Mental retardation ataxia

Silver-Russell syndrome
≠ 7q32 11p15
Growth retardation asymmetry

Beckwith-Wiedeman syndrome
≠ 11p15
Overgrowth tumor

Precocious Puberty
≠ 15q (MKRN3)
Precocious puberty

Temple s. (upd(14)mat)
≠ 14q32
Growth retardation mental retardation

Pseudohypoparathyroidism Ib
≠ 20q13.2
Parathormone resistance

Pseudohypoparathyroidism Ib
≠ 20q13.2
Parathormone resistance

Pseudohypoparathyroidism Ib
≠ 20q13.2
Parathormone resistance

Schaaf-Yang s.
≠ 15q11.2 (MAGEL2)
Neonatal hypotonia similar to PWS

Prader-Willi syndrome
≠ 15q11q13
Growth retardation mental retardation

Prader-Willi syndrome
≠ 15q11q13
Growth retardation mental retardation

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≠ 15q11q13
Growth retardation mental retardation

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≠ 15q11q13
Growth retardation mental retardation
‘Stressing’ the epigenome: glucocorticoids

Stress

Physical, psychological or environmental

Hypothalamus

CRH

Pituitary Gland

ACTH

Adrenal Glands (located above kidneys)

Cortisol

Gluccorticoid

extracellular

cytoplasm

GR

DNA helix

Nucleus

GR

C/EBP

HNF3

Ets

DRO-TF

TEF-2

Sp1

MeS-TF

Area of glucocorticoid-dependent chromatin remodeling

-2800

-2700

-2600

-2500

-2400

-2300

Glucocorticoid-dependent CpG demethylation

ηπηπππ.50σημππτοημπσγονε.χομ/?π=4772

Τημασσιν ετ ἀλ. 2001
Lots of licking and grooming of baby rat

Increased production of serotonin etc., the “happiness” neurotransmitters in the brain

Serotonin signals to the hippocampus to activate NGF-1A (Egr1)TF to acetylate histones (HAT)

HAT binds to glucocorticoid receptor gene and adds acetyl groups to histone proteins

Lower glucocorticoid secretion = ‘Chilled out rat’

Histone acetylation creates a more relaxed chromatin environment. DNA methylation is removed

Decreased DNA methylation leads to higher expression of glucocorticoid receptor

Hippocampus

$\text{Titha}$

$\text{Tithasemono}/\emptyset/\omega$ vs. $\text{antiTithasso}$
Malnourished in FIRST trimester

I.

Mary

II.

Baby normal birthweight/MtS

Baby likely to be heavier than average/ MtS

Malnourished in THIRD trimester

I.

Vicky

II.

Baby reduced birthweight/MtS

Baby normal birthweight/ no MtS

III.

Helen

Nicolas

Kelly

George
<table>
<thead>
<tr>
<th>Genes Shared</th>
<th>Relationship to Person with Schizophrenia</th>
<th>Risk of Developing Schizophrenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>Identical twins</td>
<td>Very high</td>
</tr>
<tr>
<td>50%</td>
<td>Siblings</td>
<td>High</td>
</tr>
<tr>
<td>25%</td>
<td>Grandchildren</td>
<td>Moderate</td>
</tr>
<tr>
<td>12.5%</td>
<td>First Cousins, Uncles / Aunts</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>General population</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nephews / Nieces</td>
<td></td>
</tr>
</tbody>
</table>

**US Surgeon General’s Report**
Epigenetics

Experience <-> Pathology
Sequencing

Variant Frequency

Effect Size

Variants:
- Rare
- Low frequency
- Common

Methods:
- WES
- NGS Meta-genome
- GWAS WAS
Genetics vs. Epigenetics

- Complex Systems – Stress Concepts
- Evolutionary and Developmental Stressors
- Stress and Evo-Devo
Genotype + Environment = Phenotype/Disease Phenotype

- Epigenetic control mechanisms evolve
- There is a Lamarckian dimension in evolution
- Imprints and methylation marks are erased and reestablished de novo stochastically in each generation x2
Epigenetic Regulation of Genes

**GRalpha, hippocampus, NGF-IP (stress, sexual abuse, depression)**

**BDNF, cortex (stress, sexual abuse, depression)**

**GRalpha, liver (obesity)**

**PPARalpha, liver (obesity)**

**Pdx1, islets of Langerhans (diabetes mellitus type 2)**

**ERalpha, hypothalamus (female behavior)**

**AR, brain (male behavior), skin (hirsutism)**

**FTO, (body comp., obesity) -> IRX3 homeobox transcription factor**

**Nanog, Oct 4 (neural stem cell differentiation)**

**DLK1-MEG3, (obesity, diabetes type 1 and 2)**

**LXRalpha, (obesity, carbohydrate intolerance)**

**Kisspeptin, –puberty**

**CRH, - labor and delivery**

**FKBP5, –GR function (anxiety, depression)**
Epigenetics of Retrotransposons (Piwi protein-associated ncRNAs called piRNAs)

- ~60% of genome of retroviral origin
- 10% of genome consists of Alu repeats
- 10,000 HERV-K retrotransposons
- 3,000-5,000 SVA retrotransposons
The Piwi protein-piRNA pathway provides an adaptive defense in the transposon + viral arms race

Increasingly complex networks of small RNAs act through RNA-interference (RNAi) pathways to:

- restrain the spread of “selfish” genetic elements
- mediate antiviral responses
- regulate gene expression
- organize chromosomal domains
Human endogenous retroviral element K10 (HERV-K10) is altered in *in vitro* handled human blastocysts

\[\downarrow\]

Decreases methylation of the imprinted *DLK1-MEG3* gene region on chromosome 14q32.2.

*Dimitriadou et al., Stress 2013*
Systolic and Diastolic Blood Pressure-SDS

Sakka et al. Fertility Sterility 2010
Results
Comparison between SGA-IVF, AGA-IVF and controls

Sakka et al. Fertility Sterility 2010
Triglycerides

Sakka et al. Fertility Sterility 2010

p = 0.031
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control N=42</th>
<th>ICSI N=42</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>83.7± 9.3</td>
<td>81±7.7</td>
<td>0.36</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>6.9± 6.7</td>
<td>5.5± 2.5</td>
<td>0.9</td>
</tr>
<tr>
<td>HOMA index</td>
<td>1.5± 1.8</td>
<td>1.1±0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Total Chol (mg/dL)</td>
<td>172.7±24.5</td>
<td>167.7±25.3</td>
<td>0.35</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>54.2±22.6</td>
<td>45.4±16.5</td>
<td>0.07</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>60.8±12</td>
<td>63.9±8.9</td>
<td>0.17</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>100.9±22</td>
<td>94.6±21.2</td>
<td>0.18</td>
</tr>
<tr>
<td>ApoA1 (mg/dL)</td>
<td>156.1±19.8</td>
<td>153±21.1</td>
<td>0.5</td>
</tr>
<tr>
<td>ApoB (mg/dL)</td>
<td>74.2±14.9</td>
<td>75.7±14.7</td>
<td>0.65</td>
</tr>
<tr>
<td>Lp(a) (mg/dL)</td>
<td>15.4±20.3</td>
<td>11.8±14.8</td>
<td>0.16</td>
</tr>
<tr>
<td>IGF-1 (ng/mL)</td>
<td>190.5±92.5</td>
<td>193.2±115.3</td>
<td>0.58</td>
</tr>
<tr>
<td>YKL-40 (ng/mL)</td>
<td>27.08±15.5</td>
<td>15.45±8.9</td>
<td>0.0002</td>
</tr>
<tr>
<td>hs-IL6 (pg/mL)</td>
<td>1.6± 1.5</td>
<td>2.3±4</td>
<td>0.38</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>0.78± 0.87</td>
<td>0.44±0.3</td>
<td>0.022</td>
</tr>
</tbody>
</table>

### ICSI vs. Normal Conception Children

<table>
<thead>
<tr>
<th>Increased expression</th>
<th>Decreased expression</th>
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<tbody>
<tr>
<td>Alpha-1-acid glycoprotein</td>
<td>DAN domain family member 5</td>
</tr>
<tr>
<td>Alpha-1-antitrypsin</td>
<td>Fibrinogen alpha chain</td>
</tr>
<tr>
<td>Alpha-2-HS-glycoprotein</td>
<td>Plasminogen</td>
</tr>
<tr>
<td>Alpha-2-macroglobulin</td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein A-I</td>
<td></td>
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<tr>
<td>Apolipoprotein A-IV</td>
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<tr>
<td>Apolipoprotein E</td>
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</tr>
<tr>
<td>Complement C1s</td>
<td></td>
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<tr>
<td>Complement factor B</td>
<td></td>
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<tr>
<td>Fibrinogen gamma chain</td>
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<tr>
<td>Gelsolin</td>
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<td>Haptoglobin</td>
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<tr>
<td>Keratin, type I cytoskeletal 10</td>
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<tr>
<td>Keratin, type I cytoskeletal 9</td>
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<tr>
<td>Keratin, type II cytoskeletal 1</td>
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<tr>
<td>Prothrombin</td>
<td></td>
</tr>
<tr>
<td>Serotransferrin</td>
<td></td>
</tr>
<tr>
<td>Transthyretin</td>
<td></td>
</tr>
<tr>
<td>Vitamin D-binding protein</td>
<td></td>
</tr>
</tbody>
</table>

Kosteria et al. 2017
Representation of functional classification of identified, differentially expressed plasma proteins (% total number of identified).

Kosteria et al. 2017
Environment Special:
The oceans—why 70% of our planet is in danger

Afghanistan:
After a flawed election, how the world can help

How the first nine months shape the rest of your life
The new science of fetal origins

BY ANNIE MURPHY PAUL
**Blastulation**: 1 - morula, 2 - blastula.

- Oocyte to 2 cells: 32 genes
- Two to 4 cells: 129 genes

*Tohonen et al. Nat Comm 2015*
Blastocyst

- endometrium
- inner cell mass (embryoblast)
- trophoblast
- blastocyst cavity (blastocoele)
Epigenome

- **Preimplantation: Methylome erasure**
  - Paternal first, maternal ensues
- **Remethylation: Morula-Blastocyst**
- **Inner cell mass: hypermethylation**
- **Trophoblast: hypomethylation**

- **Gametogenesis: epigenetic reprogramming/erasure-remethylation**

- "**Epigenetic Clock**" >300 genes (>80 are GR-dependent)
Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse

Patrick O McGowan¹,², Aya Sasaki¹,², Ana C D’Alessio³, Sergiy Dymov³, Benoit Labonté¹,⁴, Moshe Szyf²,³, Gustavo Turecki¹,⁴ & Michael J Meaney¹,²,⁵

Global DNA hypermethylation
Local DNA methylation changes
Changes in histone acetylation
Alterations in miRNAs

Global DNA methylation changes
Local DNA hypermethylation
Demethylation of CpGs near GRES
Altered DNA-binding of MeCP2
Changes in histone methylation
Changes in miRNA expression

Changes in DNA methylation
Histone modifications
Altered DNA-binding of MeCP2
Alterations in miRNAs
Chromatin remodeling

Prenatal life > Childhood > Adolescence > Adulthood > Late-life

Period of rapid organism-wide and tissue-specific epigenetic remodeling
Decline in epigenetic maintenance systems
Rearrangement and loss of heterochromatin

Cumulative stress-induced epigenetic changes
Stress exposure
RNAs in Epigenetic Heredity

• *Small RNAs* in sperm transmit increased sense of fear (mice).

• *Small RNAs* in sperm transmit metabolic signals (human).

• Human milk contains *exosomes* carrying *ncRNAs, such as Gas5*, which may influence metabolism and other functions in the offspring.
The GR is key in sperm-mediated epigenetics.

In the presence of GR:
- Odor stress
- GR activation
- miRs expression
- Increased stress reactivity

In the absence of GR:
- Odor stress
- GR inactivation
- ?
- miRs expression
- Unaltered stress reactivity
Perinatal Stress-Anti-stress

• Labor and Delivery
  *Physiologic vs. Caesarian section*
• Early bonding (attachment)
  *First experiences, Breast feeding vs. Bottle feeding*
• Prematurity, Postmaturity
  *Glucocorticoid Rx*
  *Critical care (multiple stressors)*
Stress Hormones with Epigenetic Functions

- CRH, Norepinephrine (?)
- Cortisol
- Inflammatory Cytokines
• Oxytocin,
• Prolactin,
• Opioid peptides
• Endocannabinoids
• Serotonin
• Dopamine

• Empathy,
• Pro-sociality,
• Moral law
• Justice
The cycles of Hierocles

- Self
- Mind & Body
- Mother, Family
- Fellow tribesmen
- Countrymen
- Mankind as a whole
«Εκ του εισοράν γίγνετ’ ανθρώποις το εράν»

“Through empathy is love generated in Man ”

Ευριπίδης, Euripides
«Την προς αρετήν εκ παίδων παιδείαν»

“Παιδεία είναι η διαπαιδαγώγηση προς την αρετή από τα παιδικά χρόνια”

“Paideia of the virtues should start in childhood”

ΠΛΑΤΩΝ (Νόμοι 643 Ε)
Plato (Laws 643 E)
Figure 2.6
(a) Rates of return to human capital investment initially setting investment to be equal across all ages

Rates of return to human capital investment initially setting investment to be equal across all ages

Carneiro and Heckman 2003
### Epigenetic Machinery Defects and Undergrowth and Intellectual Disability Syndromes

**Kabuki syndrome (AD):**

- **MML2 Gene**, *MML2* **Highlighter/writer** protein adding activating methyl groups to histone tails.

**Rett syndrome (X-linked D):**

- **MECP2 Gene** *(X chromosome)*, **Reader/decoder** Methyl CpG-binding protein 2.
Epigenetic Machinery Defects and Overgrowth and Intellectual Disability Syndromes

Sotos syndrome (AD):

Polycomb repressive complex 2 (subunits: \textit{NSD1} = \textit{histone methyltransferase}, \textit{EZH2}, \textit{DNMT3A}, \textit{CHD8}, \textit{HIST1H1E}, \textit{EED})

Weaver syndrome (AD):

Polycomb repressive complex 2 (subunits: \textit{EZH2} = \textit{histone methyltransferase}, \textit{EED}, \textit{SUZ12})
The HMGA2-PLAG1-IGF2 Pathway in Normal and Silver-Russell Cases

Normal

Silver-Russell Syndrome
The shape of things to come
Stress/inflammation

Social conditions: Inequality, Dignity, etc.

“Chronic Stress and Inflammation Syndrome” (CSIS)
Psychologic and somatic manifestations:
MUS, Anxiety, Depressive symptomatology, etc.
Obesity, Osteosarcopenia,

Sleep disorders, Accelerated Aging
ENVIRONMENTAL STRESSORS

Species vs. Individual

Evolution
Genetics
CNS complexity

Genotype

Phenotype

Development
Epigenetics
CNS plasticity

Epigenotype

Genotype

Species

ENVIRONMENTAL STRESSORS

Starvation
Dehydration
Injurious agents-inflammations
Adversaries-anticipation
Adversaries-avoidance
Injury-minimization
Social bonding disruption

Embryogenesis
Maternal Stress
Perinatal Stress
Selections of **Genetic** and **Epigenetic** Networks Participating in Functions Important for Human Survival and Species Preservation

<table>
<thead>
<tr>
<th>RESPONSE TO SURVIVAL THREAT</th>
<th>SELECTIVE ADVANTAGE</th>
<th>CONTEMPORARY DISEASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combat starvation</td>
<td>Energy conservation</td>
<td>Obesity/metabolic syndrome</td>
</tr>
<tr>
<td>Combat dehydration</td>
<td>Fluid and electrolyte conserve</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Combat injurious agents</td>
<td>Potent immune reaction</td>
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Chrousos, Amer J Med 2004
Life course

Developmental epigenetic plasticity

Inadequate response to stressors

Chronic Noncommunicable Accumulating Disease Risk

Mother and Fetus

Infant

Childhood & Adolescence

Adulthood

No intervention

Late intervention impactful for vulnerable groups

Late intervention

Earlier intervention improves functional capacity & responses to new challenges

Early intervention

Life course
Αἰὼν παῖς ἐστι παίζων πεσσεύων· παιδὸς ἢ βασιληή.

Ο Χρόνος είναι ένα παιδί που παίζει, ρίχνοντας ζάρια· ενός παιδιού είναι η βασιλεία.

Time is a child that plays dice; the kingdom belongs to a child.

Ηράκλειτος (544-484 BCE)
Herakleitos
‘Be equanimous and remember not to believe easily’
24 H SAMPLING

OVERNIGHT DEXAMETHASONE TEST

A.

PLASMA CORTISOL

24 H CORTISOL

8 am  8 pm  DARK  8 am

NS  CS

TARGET TISSUE SENSITIVITY

TARGET TISSUE RESPONSE

CORTISOL CONCENTRATION

HS  N  R  THRESHOLD FOR HARMFULNESS

NS  CS

Chrousos JCEM 1998
Results #1: Clock/Bmal1 Represses GR Transcriptional Activity through Acetylation

In the Absence of Acetylation by CLOCK

In the Presence of Acetylation by CLOCK

GR induced Transcriptional Activity

Acetylation Sites

Interaction with Clock

K480 K492 K494 K495

GR

NTD

DBDHR

LBD

420 480 520

777
Uncoupling between Circadian Rhythm of Serum Cortisol and Tissue Glucocorticoid Sensitivity

24 H SAMPLING

OVERNIGHT DEXAMETHASONE TEST

Normal
Stress
Shift

Serum Cortisol

8 pm Dark 8 am

CIRCADIAN TISSUE GLUCOCORTICOID SENSITIVITY/GR ACETYLATION

Target Tissue Glucocorticoid Sensitivity

8 am 8 pm Dark 8 am

Functional Glucocorticoid Hypersensitivity
Pathologic Consequences

GR Acetylation

Target Tissue Glucocorticoid Sensitivity

8 am 8 pm Dark 8 am
Serum cortisol levels before and through fasting month:

\[ P < 0.01 \]

NS
MUSCLE MASS

Best predictor of morbidity and life expectancy
DEFINITIONS

- Osteosarcopenia vs. Lean Paradoxic Obesity vs. Osteosarcopenic Obesity

**Decreased bone mass:**

- Osteopenia vs. Osteoporosis
  - T-scores from -1 to -2.5 vs. <2.5

**Decreased muscle mass:**

- Sarcopenia vs. Sarcasthenia (frailty)
Weight + Height

Hologic-DXA

BIA-ACC
Stress and inflammatory biomarkers and symptoms are associated with bioimpedance measures

Constantine Tsigos*, Charikleia Stefanaki†, George I. Lambrou†, Dario Boschiero§ and George P. Chrousos†,‡,¶

*School of Health Sciences and Education, Harokopio University of Athens, †Division of Endocrinology, Metabolism and Diabetes, University of Athens Medical School, “Eugenideion” Hospital, ¶First Department of Pediatrics, Choremeio Research Laboratory, University of Athens, §BIOTEKNA Co., Venice, Italy, ¶Biomedical Research Foundation, Academy of Athens, Athens, Greece
Pearson’s correlation co-efficient for BIA and DXA Fat Mass in kg & as Body Weight %
Participants were 99,571 adult Caucasians (29,624 males and 60,047 females), ages 20-80 y, grouped by:

- **BMI**: Lean, Overweight, Obese
- **Presence of Medically Unexplained Symptoms** = MUS
- **Body Composition**
The MUS symptoms examined*:

(i) persistent tiredness or fatigue,
(ii) depressive symptomatology,
(iii) persistent insomnia or night awakenings,
(iv) persistent drowsiness during the day,
(v) anxiety,
(vi) apathy,
(vii) panic attacks,
(viii) changes in heart rate (arrhythmias/tachycardia) at rest,
(ix) changes in appetite (appetite loss or excessive hunger),
(x) night binge eating,
(xi) stomach cramps, bloating or gastro-esophageal reflux disease (GORD),
(xii) presence of irritable bowel syndrome (IBS) symptoms,
(xiii) cold hands and feet,
(xiv) sweating during sleep.

*Presence of MUS was defined as a positive answer to more than 3 of the above 14 questions.
Evaluation included:

- Advanced Body Composition Analysis by BIA-ACC vs. DEXA
- Indices of Inflammation: hsCRP, Interleukin-6
- Indices of Stress: am and pm Salivary Cortisol, Delta-Cortisol (am-pm)
hsCRP Levels in Participant's Groups

Distribution of Participants with no Inflammation
no MUS (n=2750)

Distribution of Participants with Inflammation with MUS (n=74734)

Distribution of Participants with no Inflammation
no MUS with excessive FM (n=37099)
Cortisol Levels (mg/l) in Participant's Groups

- **cortisol 8am**
- **cortisol 8pm**

Distribution of Participants with no Inflammation and no MUS (n=2750)

Distribution of Participants with Inflammation with MUS (n=74734)

Distribution of Participants with no Inflammation and no MUS with excessive FM (n=37099)
Healthy Overweight/Obese Youth: Early Osteosarcopenic Obesity Features.

Stefanaki C, Peppa M, Boschiero D, Chrousos GP.

Overall, 2551 subjects (974 males) aged 18–21 years participated in the study.

**The healthy lean group** included 1072 participants [900 males (84%) and 172 females (16%)].

**The healthy overweight/obese group** included 1479 participants [74 males (5%) and 1405 females (95%)].
$F(3,2547) = 2824.545, p<0.001, \text{Eta squared } = 0.76$
F(3, 2547) = 935.110, p<0.001, Eta squared = 0.52
F(3, 2547) = 10.901, p < 0.001, Eta squared = 0.012
F(3,2547) = 368.272, p<0.001, Eta squared =0.3
F(3, 2547) = 2824.545, p < 0.001, Eta squared = 0.76
F(3, 254) = 793.640, p < 0.001, Eta squared = 0.48

p = 0.99
$F(3,2547) = 892.223, \ p < 0.001, \ \eta^2 = 0.51$

$p = 0.99$

$F(3,2547) = 892.223, \ p < 0.001, \ \eta^2 = 0.51$
$F(3,2547) = 554.67$, $p < 0.001$, $\eta^2 = 0.39$
$\text{F}(3,2547) = 1679.870, \ p<0.001, \ \text{Eta squared} = 0.66$
CONCLUSIONS

• Osteosarcopenic phenotype is common and exists even in the young, suggesting early start of prevention and treatment.

• “Healthy” lean, overweight or obese populations may demonstrate:
  1. Decreased bone mass;
  2. Decreased muscle mass;
  3. Increased hsCRP concentrations;
  4. Flattening of cortisol circadian rhythm;
  5. MUS

• BIA-ACC is a highly potent device that may detect osteosarcopenic phenotypes, and may be used for early intervention.

• Future cohort studies are needed to establish the definite causative factors behind the negative relations between fat, bone & muscle mass.
Genetic vs. Epigenetic Diseases

- Gene Effect Size
- Age at Onset
Relative unrooted Pol neighbor joining (NJ) dendrogram
Deming plot with fat mass in kg

\[ y = -4.7357 + 1.0396 \times \]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient</th>
<th>S.E.</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-4.7357</td>
<td>1.3244</td>
<td>-7.3699 to -2.1015</td>
</tr>
<tr>
<td>Slope</td>
<td>1.0396</td>
<td>0.04561</td>
<td>0.9489 to 1.1303</td>
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Grouping:

• 10,416 lean subjects (8,810 males) with no MUS (Group A),

• 58,710 lean subjects (5,581 males) with MUS (Group B), and

• 30,445 overweight/obese subjects (15,133 males) with no MUS (Group C).
Participants were 99571 adult Caucasians (29624 males and 60047 females), ages 20-80 y, grouped by:

- **BMI**: Lean, Overweight, Obese
- **Presence of Medically Unexplained Symptoms** = MUS
- **Correlation with body composition parameters**
Extracellular Water (%) in Participant's Groups

- Distribution of Participants with no Inflammation no MUS (n=2750)
- Distribution of Participants with Inflammation with MUS (n=74734)
- Distribution of Participants with no Inflammation no MUS with excessive FM (n=37099)
Somatic Growth vs. Psychological Development

Growth: *Embryonic growth, Stature, Body weight, Body composition, Body shape, Puberty*

Development: *Psychologic Maturation*
Genetics vs. Epigenetics

- Complex Systems
- Evolutionary and Developmental Stressors
- Stress and Evo-Devo
Experimental Results on the Interactions of Clock/BMAL1 and GR (1)

Clock/Bmal1 Represses GR-induced Transcriptional Activity

GR Interacts with Clock (371-854) in GST Pull-down Assays

Activity is HAT-dependent

Clock Acetylates Multiple Lysines in the GR Hinge Region

Multiple Lysines in the GR Hinge Region are Necessary for Clock to Repress GR Transcriptional Activity

Clock/Bmal1 Knockdown Enhances GR-induced Transcriptional Activity
Experimental Results on the Interactions of Clock/BMAL1 and GR (2)

Clock Reduces Binding of GR to GREs in vitro

Clock/Bmal1 Reduces the GR/GRE Binding in vivo (ChIP Assays)

Circadian mRNA Expression of CLOCK-related Genes in PBMCs in vivo

GR is Acetylated in a Circadian Fashion in PBMCs in vivo

Gene-specific Circadian mRNA Expression of Glucocorticoid-responsive Genes in PBMCs in vivo (Transactivation)
Experimental Results on the Interactions of Clock/BMAL1 and GR (3)

Gene-specific Circadian mRNA Expression of Glucocorticoid-responsive Genes in PBMCs in vivo (Transrepression)

Circadian mRNA Expression of CLOCK-related Genes in PBMCs in vivo

Clock and GR are Associated with Each Other in PBMCs ex vivo

GR Acetylation is Dependent on the Presence of Clock in PBMCs ex vivo

Clock Knockdown Abolishes mRNA Fluctuation of Glucocorticoid-responsive Genes in PBMCs ex vivo
Social conditions: Inequality, Dignity, etc.

Stress/inflammation

Sleep disorders, Accelerated Aging

“Chronic Stress and Inflammation Syndrome” (CSIS)

Psychologic and somatic manifestations:
Obesity, Osteosarcopenia, MUS, Anxiety, Depressive symptomatology, etc.
ENVIRONMENTAL STRESSORS

Species vs. Individual

Evolution
Genetics
CNS complexity

Genotype

Phenotype

Development
Epigenetics
CNS plasticity

Epigenotype

Starvation
Dehydration
Injurious agents-inflammations
Adversaries-anticipation
Adversaries-avoidance
Injury-minimization
Social bonding disruption

Embryogenesis
Maternal Stress
Perinatal Stress
Selections of **Genetic** and **Epigenetic** Networks Participating in Functions Important for Human Survival and Species Preservation

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<th>RESPONSE TO SURVIVAL THREAT</th>
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<th>CONTEMPORARY DISEASE</th>
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