The Role of DNA methylation in the link between adversity and child and adolescent psychopathology

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Genetic and ontogenetic determinants of adult behavior in the rat

Infantile Experience and Resistance to Physiological Stress

It has been reported (1) that rats which had been "gentled" (that is, picked up and stroked once daily for 21 days postweaning) were heavier and reacted with less cardiovascular and gastrointestinal damage under conditions of immobilization for 48 hours than did the "nongentled" controls. In addition, adrenal glands of the nongentled were heavier than those of the gentled animals. Subsequent research (2) has revealed that rats handled prior to weaning showed significantly less mortality following 5 days of total food and water deprivation than did nonhandled rats or rats handled after weaning. The present experiment (3) was designed to investigate the response of rats, handled and nonhandled, in infancy (days 1 through 20) to physiological stress.

Figure 1. Comparison of weights of adrenals of the various groups. The lengths of the bars indicate the range of individual values; the light lines, the means; and the numbers, the subjects within each group.

1957/63
1996
2004

Early experiences have profound effects on later mental and physical health
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Quality of early parent child interactions

- Meaney & colleagues
  - Arched back nursing + licking & grooming (90%)
Quality of early parent child interactions

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  - Arched back nursing + licking & grooming (90%)

Epigenetic programming by maternal behavior

Ian C G Weaver¹,², Nadia Cervoni³, Frances A Champagne¹,², Ana C D’Alessio³, Shakti Sharma¹, Jonathan R Seckl⁴, Sergiy Dymov³, Moshe Szyf²,³ & Michael J Meaney¹,²
DNA methylation = the mechanism

• DNA methylation is an epigenetic mechanism that regulates **gene expression**.

• Addition of **methyl molecule** to DNA base pairs

• **Rule of thumb:**
  • **higher** DNA methylation = **lower** gene expression
DNA methylation in living humans?

- **Environmental input is important**
  - Fraga et al. (2005), Compared identical twins
  - Age range: 3y – 74y
  - Similar epigenomes in childhood
  - Very different in adulthood
DNA methylation in living humans?

- Environmental input is important
  - Fraga et al. (2005), Compared identical twins
    - Age range: 3y – 74y
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    - Very different in adulthood
• **Also influence levels of DNA methylation**
  – After embryonic development
  – As early as birth (cord blood)
  – <30% DNAm sites are influenced by genetic polymorphisms
    • Likely an underestimate (!!)

![Box plot showing average methylation (%) at three adjacent CpG sites](image)
Animal vs human studies

• **Animals**
  – **MECHANISM** (Causal; Mediator; Reversal)
    • Hippocampus (CNS)
    • Functional characterisation (expression)
    • Genetic and environmental components

• **Children and adolescents (living)**
  – **BIOMARKER** (??)
  – Peripheral samples (i.e. blood, cheek swabs)
  – May not be surrogate of central nervous system
  – May not be mechanism of disease aetiology

The ‘promise’... mediational framework

DNA Cytosine Methylation

$\text{Unmethylated}$

$\text{Methylated}$

$\rightarrow$ Gene Expression

$\rightarrow$ Gene Expression Repressed

$a$ path

$\rightarrow$ $a*b$ path

$b$ path

Adversity

Ideas about the future

Annual Research Review: DNA methylation as a mediator in the association between risk exposure and child and adolescent psychopathology

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State of the scientific literature

Published studies (2000-2016)

- DNAm + Exposure (n tot = 919)
- DNAm + Exposure + Child* (n tot = 170)
- DNAm + Exposure + Child* + Longitudinal (n = 21)
- DNAm + Psych* (n tot = 400)
- DNAm + Psych* + Child* (n tot = 129)
- DNAm + Psych* + Child* + Longitudinal (n = 15)

‘a path’

- Stress
- Nutrition
- Neurotoxic

a path: Adversity → DNA methylation

- **Glucocorticoid receptor (NR3C1)**
  - Anxiety/Depression
    (Hompes et al., 2013; Oberlander et al., 2008)
  - Maternal mental health & ‘stroking’
    (Murgatroyd et al., 2015)
  - Remember: **STRESS RESPONSE**

- **Dutch hunger winter** (Heijmans et al. 2008)
  - Sibling design (60 years later)
  - IGF2 DNA methylation

- **Natural experiment** (Kumsta et al., 2016)
  - Romanian adoption study
  - Extended/Limited/UK matched non-exposed
  - CYP2E1 Region: 9 sequential CpGs
  - Social cognition

Candidate vs Genome-wide

**ANALYTICAL METHODS**

**CANDIDATE**
- Focus on DNAm in individual, preselected genes (typically one)
- Based on a priori hypotheses

**EPIGENOME-WIDE**
- Epigenome-wide association studies (EWAS)
- Investigate thousands of DNAm markers across the genome
- hypothesis-free

a path: Child Maltreatment

- **Childhood maltreatment**
  - Abuse, neglect
  - Candidate gene & retrospective
    - *NR3C1, SLC6A4, FKB5, BDNF*
    - Lutz & Turecki, 2014; Turecki & Meaney, 2016

- **Yang et al. (2013) EWAS**
  - N = 96 `+` and 96 `-` (matched)
  - 2,686 loci, *CCDC85, PTPRN*
  - Physical and psychiatric problems

- **Cecil et al. (2016) EWAS**
  - N = 124, high risk youth
  - Physical maltreatment (e.g. *GABBR1*)
  - Unique and common methylation

- **Limitations**: Cross-sectional (reverse causality)
  - Yang and Cecil did not overlap (Saliva)
  - sample characteristics, ages
1. DNAm has been associated with a range of pre-and postnatal adversities.
   
a) Prenatal studies have generally been prospective
b) Postnatal studies generally been retrospective and/or cross-sectional

2. To test methylation as a mediator, the risk exposure should come before methylation

3. Handful of studies have replicated findings, used negative controls or integrated genetic information to strengthen causal inference

4. Mechanism or biomarker?

'b path'

- Wide range
- Externalising

Adversity

DNA Cytosine Methylation

Path a

Path b
Focus on **NR3C1** and early neurobehavioural profiles

- Placenta methylation associate with reactivity and excitability in new-borns (Appleton et al., 2014; Paquette et al., 2015)
- Cortisol response and self-regulation after social stressor in 5 mos olds (Conradt et al., 2015)

**SLC6A4**: Placental ~ negative emotionality and temperamental difficulties in preterm infants (Montirosso et al., 2016a,b)

- Soothability exposed to SSRIs (Garstein et al., 2015).

**EWAS**: Placenta *FHIT* and *ANKRD11* (Paquette et al 2016)

- Attention, lethargic behaviour, movement quality and arousal
b path: Internalising problems

• Also high focus on **NR3C1**

• ... associated with internalizing –not externalizing –problems (Parade et al 2016)

• .... Morning cortisol levels (Dadds et al. 2015).

• **EWAS:** in 18 MZ twin pairs discordant for depression
  – **STK32C**
  – associated with depression post-mortem cerebellum (Dempster et al. 2014)

Inconsistencies in Candidate vs EWAS

• **NR3C1**
  – Well characterised gene in terms of function
  – Implicated in wide range of developmental and metabolic functions

• **Does not emerge in EWAS**
  – likely to be of small effect size (see Weder 2014; Cecil et al. 2017)

• EWAS studies *themselves* do not replicate (!!!)
  – Many small samples
  – EAGLE: consortia

**b path: Externalising problems**

- Larger number of studies: Aggression, CP, ADHD, ODD

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**Neonatal DNA methylation and early-onset conduct problems: A genome-wide, prospective study**

CHARLOTTE A. M. CECIL, ESTHER WALTON, SARA R. JAFFEE, TOM O’CONNOR, BARBARA MAUGHAN, CAROLINE L. RELTON, REBECCA G. SMITH, WENDY MCARDLE, TOM R. GAUNT, ISABELLE OUELLET-MORIN, AND EDWARD D. BARKER

King’s College London; University of Bristol; University of Pennsylvania; University of Rochester Medical Center; Exeter University; and University of Montreal
b path: *Externalising problems*

- Larger number of studies: Aggression, CP, ADHD, ODD

Cecil, Walton…..Barker (in press) *Development & Psychopathology*
b path: Externalising problems

- Larger number of studies: Aggression, CP, ADHD

Cecil, Walton…..Barker (in press) Development & Psychopathology
• Candidate gene = Dopamine & Serotonin signalling

• EWAS: Walton et al., 2016
  – Propsective (ALSPAC)
Epigenetic profiling of ADHD symptoms trajectories: a prospective, methylome-wide study

E Walton¹,6, J-B Pingault²,3,6, CAM Cecil¹, TR Gaunt⁴, CL Relton⁴, J Mill³,⁵ and ED Barker¹
Epigenetic profiling of ADHD symptoms trajectories: a prospective, methylome-wide study
b path: Comorbidity of ADHD and ODD

Walton et al. (2016) *Molecular Psychiatry*

- 108 genes shared (cord blood at birth)
- Protocadherin family (cell-cell connections in brain)
- Borghol et al. 2012: 1958 British Cohort Study

Barker, Walton, Cecil et al. (in press) *Child Development*
$b$ path: Summary

• Suggestive that $b$ path is possible
  – Retrospective/few replications
  – Few account for genetic influence
  – Only handful biologically characterise methylation
    • Statistical vs biological significance

Walton, Cecil ... Barker (2017) *Journal of Child Psychology and Psychiatry*
The ‘promise’… mediational framework

Can DNAm shed light on substance use liability?

- Individuals vary in their susceptibility to substance use and addiction risk
  - Can we identify early epigenetic predictors?
  - Are they influenced by genetic/environmental factors?
  - Does DNAm mediate environmental effects on substance use?
**Aim:** Epigenome-wide, prospective study of substance use risk

- **Prenatal influences**
  - Maternal substance use
    - Single items, 1st trimester
    - Smoking, Alcohol use
  - Maternal adversity
    - Cumulative scores, 18-32wk; Cecil et al., 2014
    - Life events, Contextual risks, Parental risks, Interpersonal risks

DNAm measured before substance use
Substance Use

Epigenetic variation at birth

- 65 FDR-corrected sites (n = 60 genes; A)
- Tightly interconnected genetic network (C)
- Enriched for neurodevelopmental processes (D)
- Certain degree of genetic influence
Genetic and environmental influences

Genetic influences

- 5/65 Likely strong genetic influence
  - Greater temporal stability

Environmental influences

<table>
<thead>
<tr>
<th>Prenatal exposures</th>
<th>Maternal smoking</th>
<th>Maternal alcohol use</th>
<th>Maternal risks</th>
<th>Family risks</th>
<th>Contextual risks</th>
<th>Life Events</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
<td>r</td>
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</tr>
<tr>
<td>Cumulative DNAm risk (birth)</td>
<td>0.20</td>
<td>1.21E-03</td>
<td>-0.07</td>
<td>0.28</td>
<td>0.15</td>
<td>0.01</td>
</tr>
<tr>
<td>Substance use (age 14-18)</td>
<td>0.32</td>
<td>1.58E-07</td>
<td>-0.03</td>
<td>0.60</td>
<td>0.32</td>
<td>1.44E-07</td>
</tr>
</tbody>
</table>

SEM model to test mediation
(prenatal risks $\rightarrow$ DNAm $\rightarrow$ substance use)
Indirect effect of prenatal risks on later substance use, via DNA methylation (DNAm) at birth?

Mediation remained after adjusting for genetic influence.
• $a$ and $b$ paths appear plausible in living children

• $a*b$ paths also

• All studies are correlational

• Models examining causal mediational pathways in living children and adolescents have yet to be published (Binder & Michaels, 2013)
The ‘promise’... mediational framework

5 ways to improve DNAm knowledge

Thoughts on future

(1) Characterising environmental effects

- Focus on single events ...

- Part of wide-range of often correlated risks
  - Depression and Nutrition (Barker et al., 2013, 2015)
  - In some cases, association with one could be proxy for other (Monk et al., 2013)
  - GR responsive to both nutrition and stress (Drake et al. 2012)
(2) Genetic Influence

- Plenty of excitement about environment
- Often do not account for genetic effects
- The genetic component may have an important role in the development of complex traits (Gaunt et al., 2016; Hannon et al., 2016)

Improved knowledge of methylation

- **Variability**: highly dynamic (peripheral samples)
  - Sex, age, tissue and cell type (Liang & Cookson, 2014)
  - What is normative? What is abnormal?

- **Cross tissue comparisons**
  - Cord blood vs whole blood vs buccal cells vs brain
  - Tissue specific vs global DNA effects

- **Scale**: Illumina 450k (very popular)
  - 2% of epigenome (though good sampling of annotated genes)
  - Illumina EPIC (900k)

• Presence of association does not imply functional effect
  – Does statistical significance overlap with functional significance?
  – Is there an impact on downstream biology?

• Additional omics
  – Using animal models
  – In vitro experiments (e.g. cell cultures)
  – In-vivo neuroimaging data (Walton et al., 2017)
  – Temporal specificity (Cecil et al., 2017; Rijlaarsdam et al., 2017)
Functional significance of loci

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(5) Strengthening causal inference

• **Strategies:**
  – Natural experiments (Mill & Heijmans, 2014)
  – Negative control (Sharp et al., 2015)
  – Mendellian epigenetic randomization (Relton & Davey Smith, 2012)
  – Clinical Trials!!
    • Response not necessarily mechanistic (biomarker)
    • In *vivo* & *vitro* experiments

Summing it up

- DNA methylation is a promising molecular mechanism by which the environment can increase risk for psychopathology
- Must be mindful to manage expectations
- **Three ways** in which epigenetic research may contribute to the understanding, prevention, and treatment of psychopathology

(1) Short-term

• Findings may be used to refine existing models of how environmental influences become biologically embedded, shift developmental trajectories and/or create latent vulnerability for psychopathology

• Developmental Origins of Health and Disease
  – Vulnerability established early
  – Potentiated thereafter

Developmental Origins of Health and Disease

- Vulnerability established early
- Potentiated thereafter

(2) Medium-Term

- As the number of replications grow and robust associations are identified...

- Epigenetic variation in specific genes may be used across clinical and research settings as biomarkers for environmental exposures, psychopathology risk, and response to treatment

(3) Long-term

• The comparison of methylation pre- vs post-intervention (e.g. via environmental enrichment, psychological therapy, medication)...

• could lend insights into the potential reversibility of psychopathology-related patterns and how best to promote resilience (Ding et al., 2016; Roberts et., 2014)

Early experiences have profound effects on later mental and physical health.
The Triangle

- Genetics // Epigenetics
- Brain (function, structure)

Behaviour // Emotions

Interdisciplinary Research
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