Before Conception: Impact of Exposure on the Methylome

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PEPH Webinar Series
Windows of Susceptibility

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The Developmental Origins of Health and Disease (DOHaD)

The environment encountered during early development can lead to changes in gene regulation that may be somatically heritable and increase risk of later adverse outcomes.
Dutch Hunger Winter

- schizophrenia
- atherogenic plasma lipids
- heightened stress response
- 3X risk of CVD

Nutr Rev. 1994 52(3):84-94
Reprod Toxicol. 2005 20(3):345-52

400-800 calories/day
The Developmental Origins of Health and Disease (DOHaD)

Most DOHaD-related studies focus on the in utero environment
Can the father pre-conceptionally impact DOHaD?
Epigenetics – DNA Methylation

**Methylome**: the entirety of the DNA methylation profile across the genome
Preconception epigenetic vulnerability: reprogramming in gametes

Most, but not all methylation reprogrammed before spermiogenesis

“Escapees” are regions that are not erased during gametogenesis
Cell 161, 2015, 1453–1467
Paternal obesity and methylation in cord blood
Hypothesis: Obesity adversely affects the fidelity of methylation reprogramming during male gametogenesis (hormone levels, endocrine disruptors)

46 men
BMI < 25

23 men
BMI ≥ 25

Bisulfite Pyrosequencing
Imprinted genes
Clin Epigenetics. 2016 6;8:51

Human Methylation 450 Beadchip
486,000 CpG sites

Endocrine Disruptors
Chlorinated organophosphates
Environ Sci & Technol Lett (In press)

Hormone Levels
Estrogen, Testosterone, Prolactin, T3, T4
Discovery, independent validation

Limitation: because beadchips and pyrosequencing are designed to analyze a population of DNA molecules, they cannot provide information about methylation profiles of individual alleles.
**TBCD**

*tubulin folding cofactor D*

Tubulin folding protein – involved in first step of folding

- strict dependence on appropriate levels of expression during development
- microcephaly, intractable seizures and developmental delay
  

- infantile neurodegenerative disorder
  

- early onset encephalopathy
  

By 450k, obese had 7% lower methylation at the CpG site shown in red (intragenic methylation)
**SOGA1**

**suppressor of glucose by autophagy**

Regulates autophagy by playing a role in glucose production

- candidate gene for feed efficiency in pigs
  - Front Genet. 2014 Sep 9;5:307

- downstream effector of adiponectin: lowers glucose production

- differentially methylated in human placenta in association with energy homeostasis

By 450k, obese had 6% lower methylation
TOBACCO
Tobacco smoke exposure

Tobacco Smoke Extract
0.1% TPM

14 days

Sperm
N=6 per group

Reduced Representation
Bisulfite Sequencing

Tobacco Smoke Extract
0.2 mg/kg/day

12 days

Sperm
N=12 per group
Paternal zebrafish genome is highly methylated; large effects
Sperm, but Not Oocyte, DNA Methylome Is Inherited by Zebrafish Early Embryos

Lan Jiang, Jing Zhang, Jing-Jing Wang, Lu Wang, Li Zhang, Guoqiang Li, Xiaodan Yang, Xin Ma, Xin Sun, Jun Cai, Jun Zhang, Xingyu Huang, Miao Yu, Xuegang Wang, Feng Liu, Chung-I Wu, Chuan He, Bo Zhang, Weimin Ci, and Jianguo Liu

CpG Site p<0.01

Methylation

Control  Exposed

http://dx.doi.org/10.1016/j.cell.2013.04.041
Large magnitude effect sizes

DarkKatze, reddit.com
CANNABIS
Cannabis influence on DNA methylation in human sperm

- 12 regular users of Cannabis, verified biologically
- 12 age-matched never-user controls
- Blood testing verified non-use of other drugs, including tobacco
- Semen specimens: semen analysis, DNA extraction
- Reduced Representation Bisulfite Sequencing (RRBS)
Cannabis use: widespread hypomethylation in sperm
# Functions of hypomethylated genes

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<tr>
<th>Term</th>
<th># Genes</th>
<th>P-value</th>
<th>Benjamini</th>
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<tr>
<td>homophilic cell adhesion</td>
<td>16</td>
<td>1.8e-8</td>
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<td>cell morphogenesis involved in differentiation</td>
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<td>neurogenesis</td>
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Functions of hypermethylated genes

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<tr>
<td>protein amino acid phosphorylation</td>
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# Acknowledgments

Special thanks to all participants in the NEST, TIEGER and Cannabis studies!

<table>
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<tr>
<th>Human (obesity)</th>
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<th>Zebra Fish (tobacco)</th>
<th>Human (Cannabis)</th>
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<tr>
<td>Sanaz Keyhan</td>
<td>Lisa Guo</td>
<td>Kelly Tomins</td>
<td>Julia Schechter</td>
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