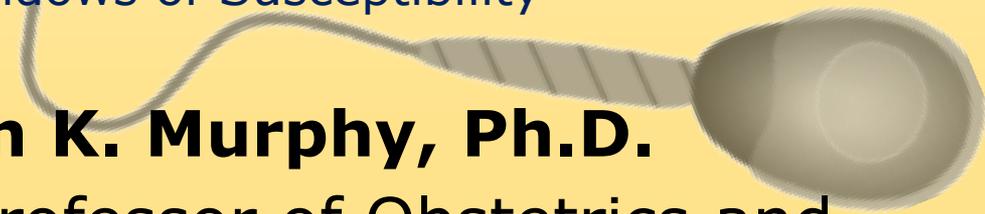


Before Conception: Impact of Exposure on the Methylome

March 8, 2017
PEPH Webinar Series
Windows of Susceptibility



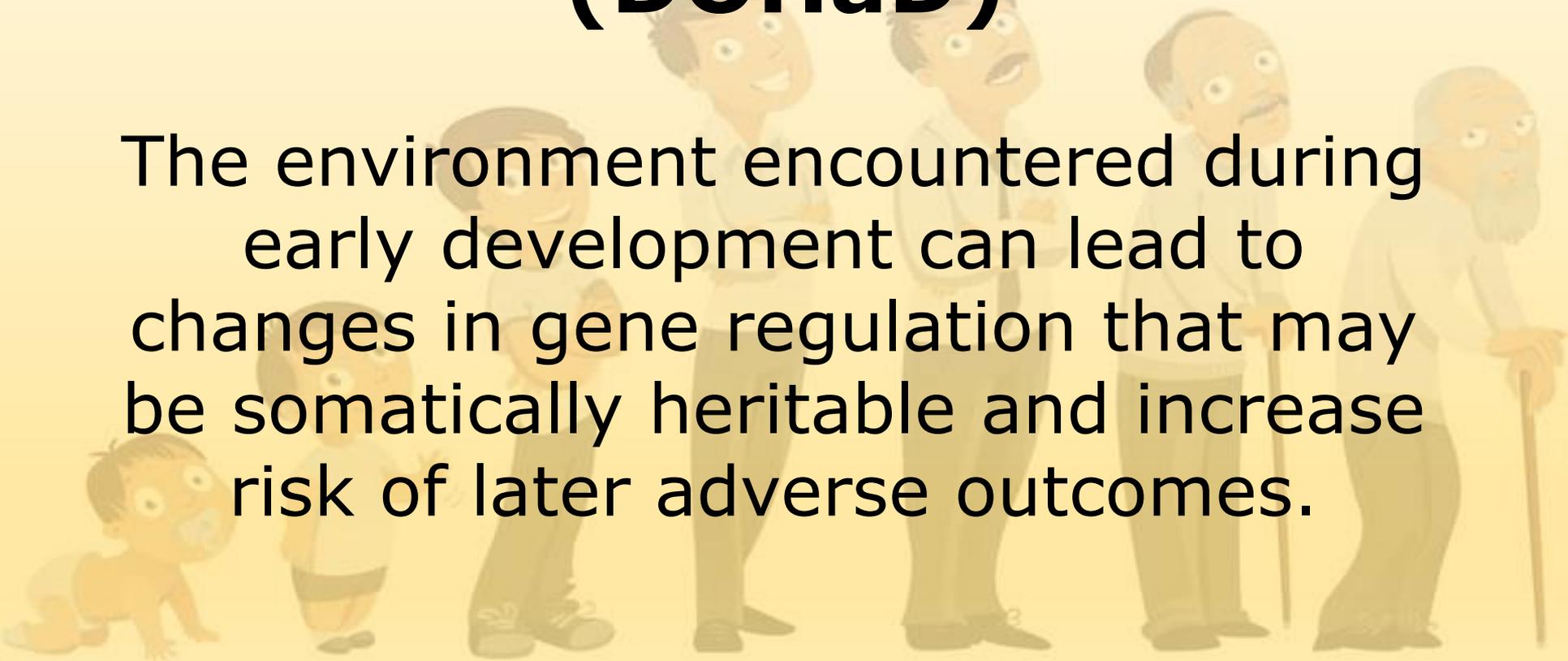
Susan K. Murphy, Ph.D.

Associate Professor of Obstetrics and
Gynecology

Duke University Medical Center

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

BROOD 48^{A(4)} **BROOD** 47^{A(4)} **BROOD** 46^{A(4)} **BROOD** 45^{A(4)} **BROOD** 48^{A(4)} **BROOD** 47^{A(4)} **BROOD** 48^{A(4)} **BROOD** 47^{A(4)} **BROOD** 46^{A(4)} **BROOD** 45^{A(4)}

Dutch Famine Study

Nederlands Home The study Results Publications Columns Links Contact

The Dutch famine birth cohort study

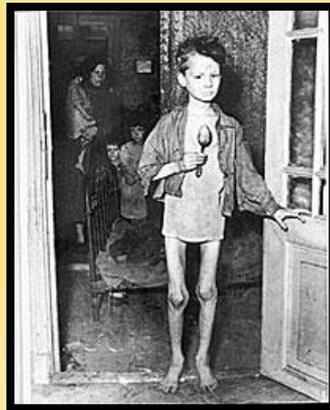
The Dutch famine birth cohort study consists of 2414 term singletons born alive between November 1943 and February 1947 in the Wilhelmina Gasthuis in Amsterdam for whom we had detailed birth records. Since 1996, the cohort has been investigated to study the effects of prenatal exposure to famine on later health.

amc
Academisch Medisch Centrum

© 2012 The Dutch famine birth cohort study
Design: ideesther.nl



400-800 calories/day



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

- decreased cognitive performance
- breast cancer
- obesity

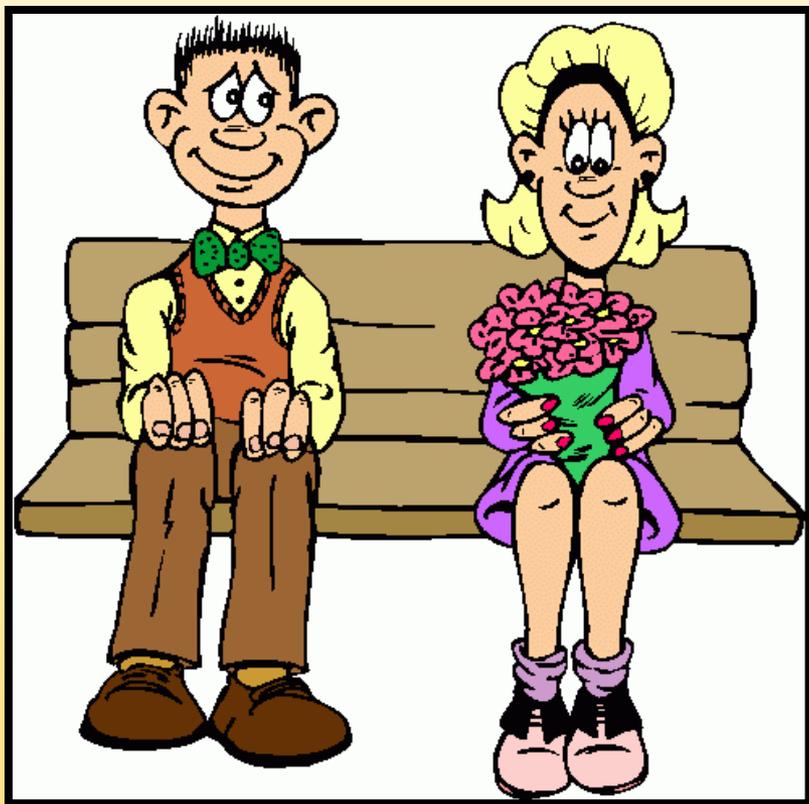
Nutr Rev. 1994 52(3):84-94
Mol Cell Endocrinol. 2001 185(1-2):93-8
Reprod Toxicol. 2005 20(3):345-52

The Developmental Origins of Health and Disease (DOHaD)



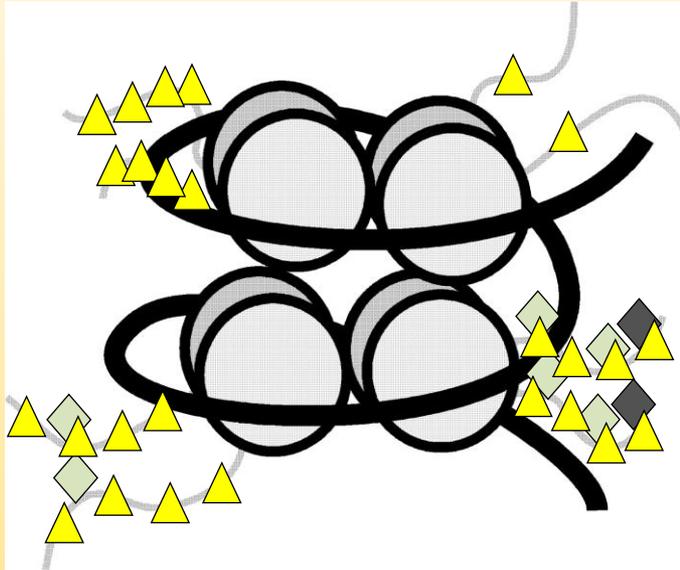
*Most DOHaD-related studies focus on
the in utero environment*

?

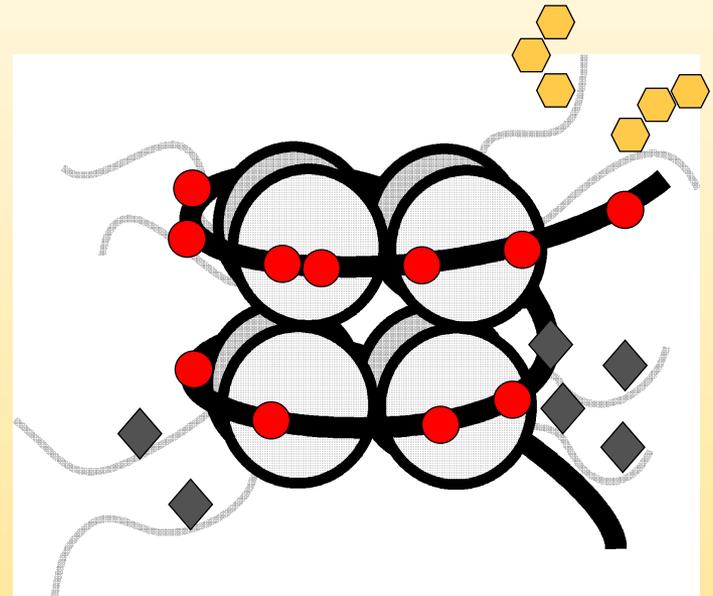
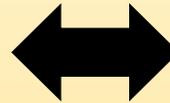


**Can the father
pre-
conceptionally
impact
DOHaD?**

Epigenetics – DNA Methylation

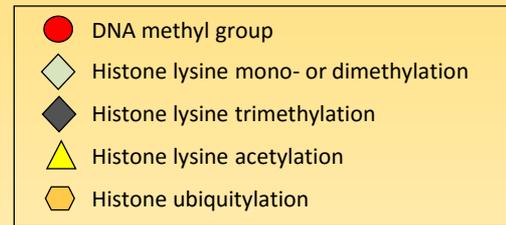


Permissive State



Repressive State

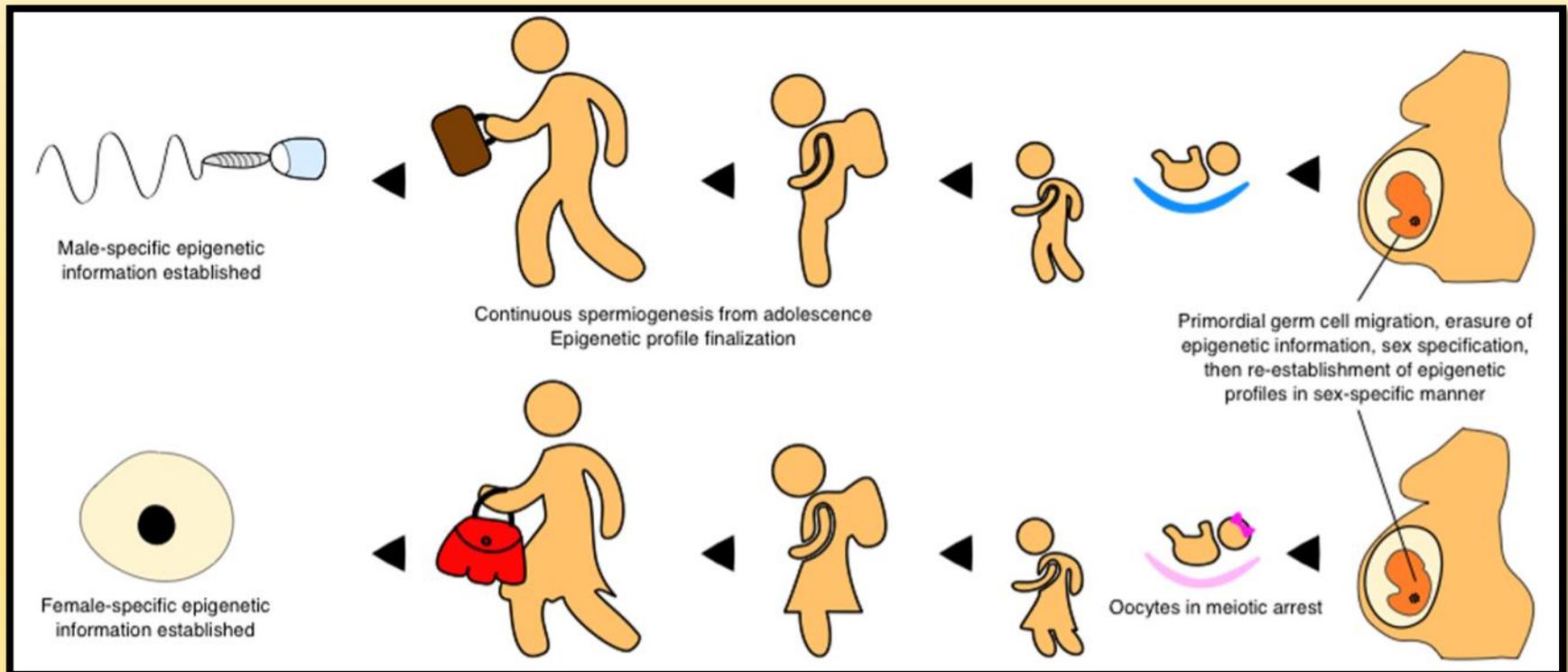
Methylome: the entirety of the DNA methylation profile across the genome



Preconception epigenetic vulnerability: reprogramming in gametes

Most, but not all methylation reprogrammed before spermiogenesis

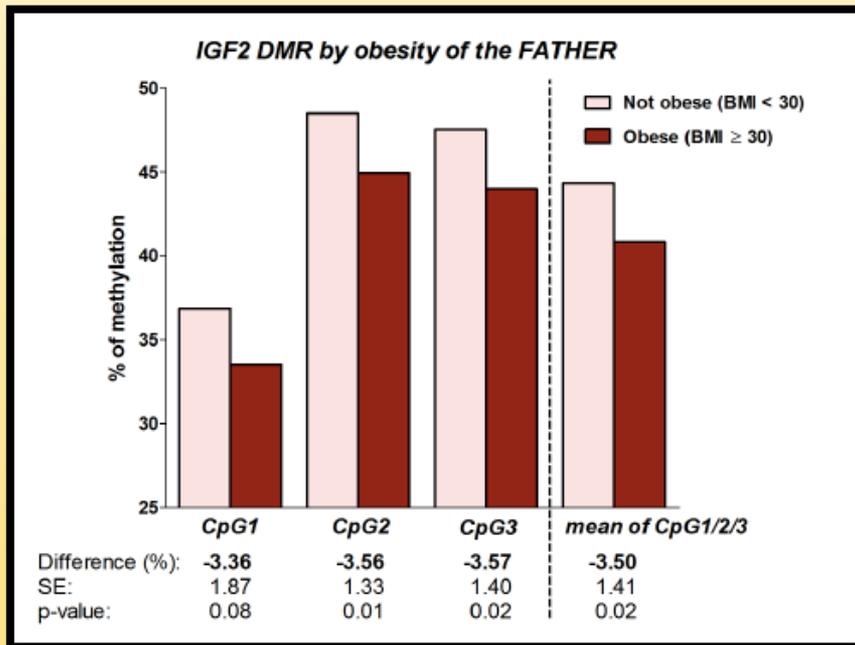
“Escapees” are regions that are not erased during gametogenesis
Cell 161, 2015, 1453–1467



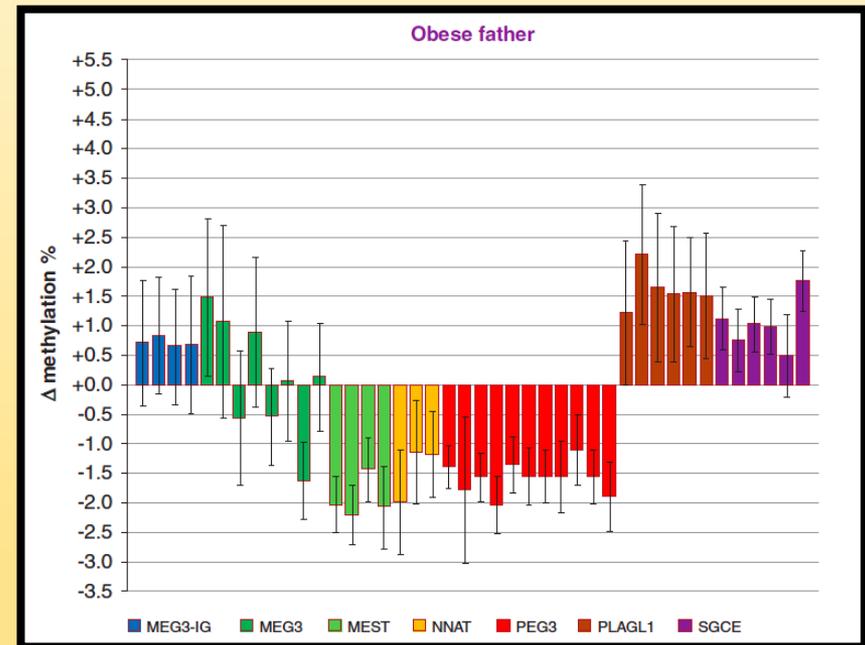


OBESITY

Paternal obesity and methylation in cord blood



BMC Med. 2013 11:29

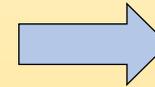
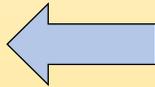


Int J Obes 2015 39:650-7

Humans Obesity and Epigenetic Reprogramming

Hypothesis: *Obesity adversely affects the fidelity of methylation reprogramming during male gametogenesis (hormone levels, endocrine disruptors)*

46 men
BMI <25



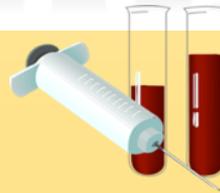
23 men
BMI ≥25



Bisulfite Pyrosequencing
Imprinted genes

Clin Epigenetics. 2016 6;8:51

HumanMethylation450 Beadchip
486,000 CpG sites



Hormone Levels

Estrogen, Testosterone, Prolactin, T3, T4

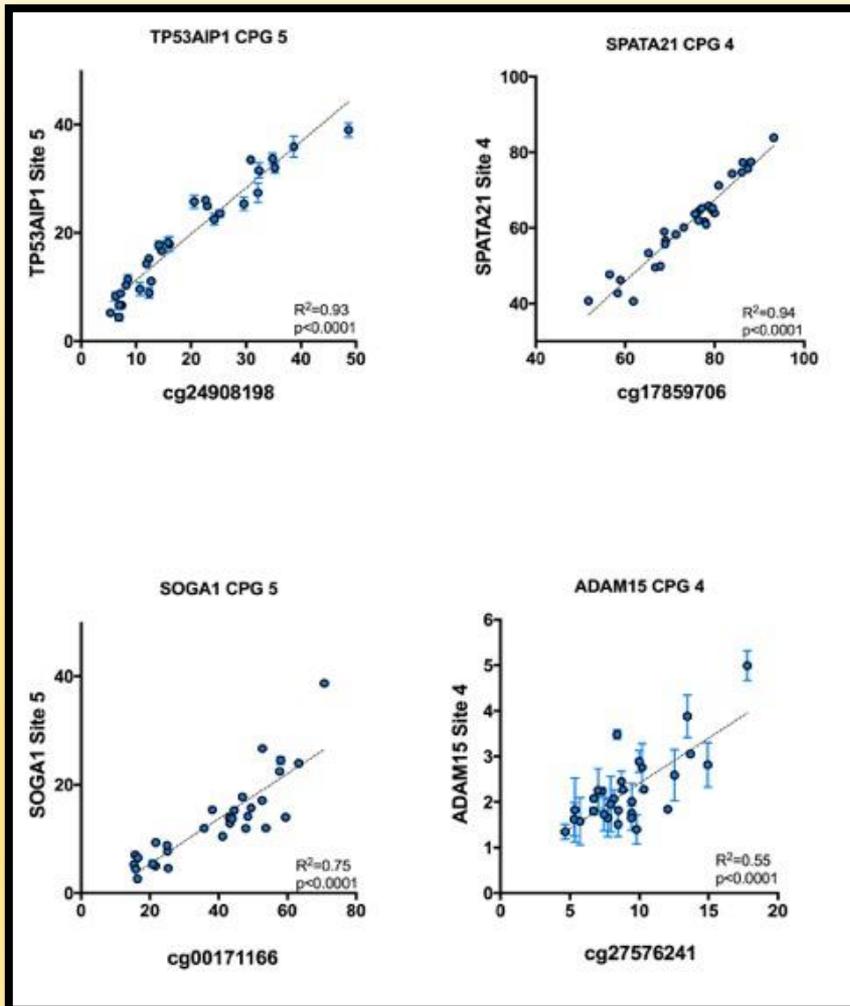


Endocrine Disruptors

Chlorinated organophosphates

Environ Sci & Technol Lett
(*In press*)

Discovery, independent validation



x-axis – 450k values
y-axis – pyrosequencing values

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

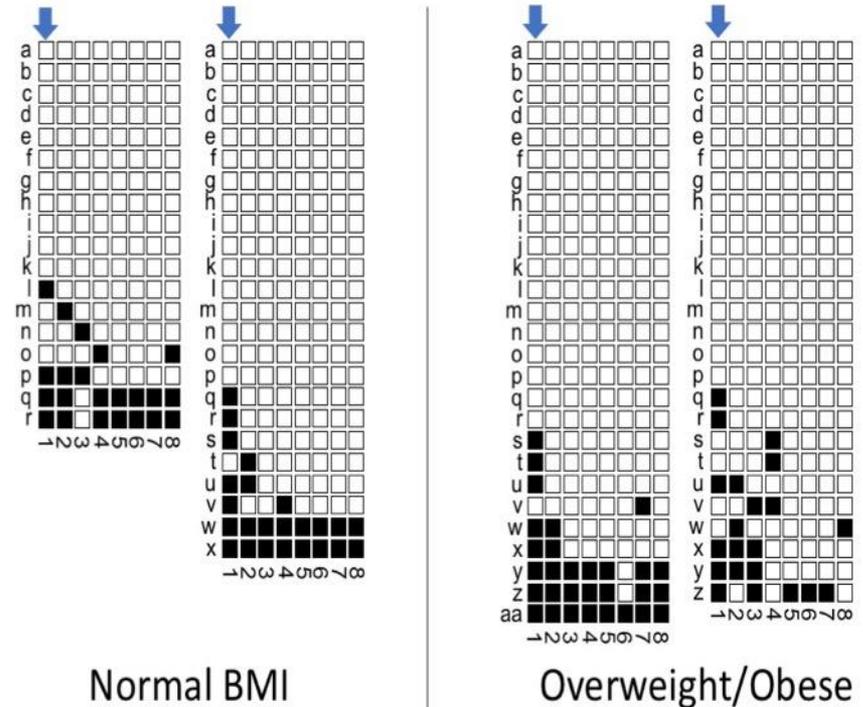
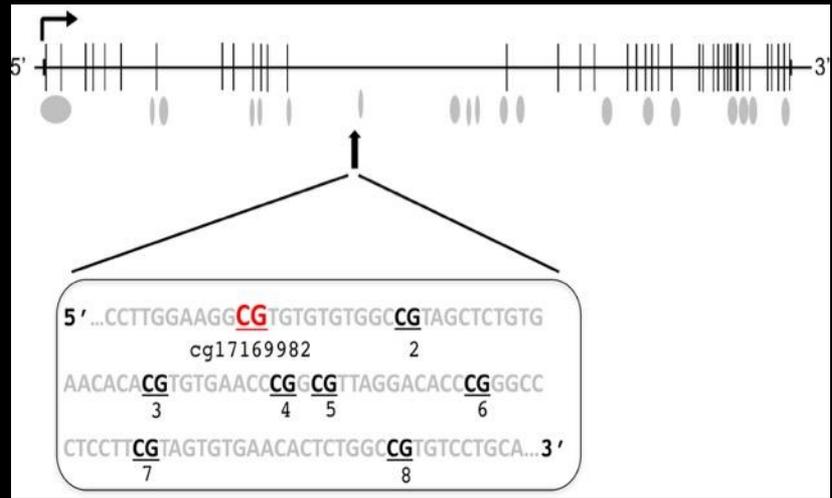
TBCD

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
Clin Genet. 2016 Nov 2. doi: 10.1111/cge.12914
- infantile neurodegenerative disorder
Hum Mol Genet. 2016 Aug 29. pii: ddw292
- early onset encephalopathy
Am J Hum Genet. 2016 Oct 6;99(4):962-973.

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

Asian-Australas J Anim Sci. 2016 Oct 28

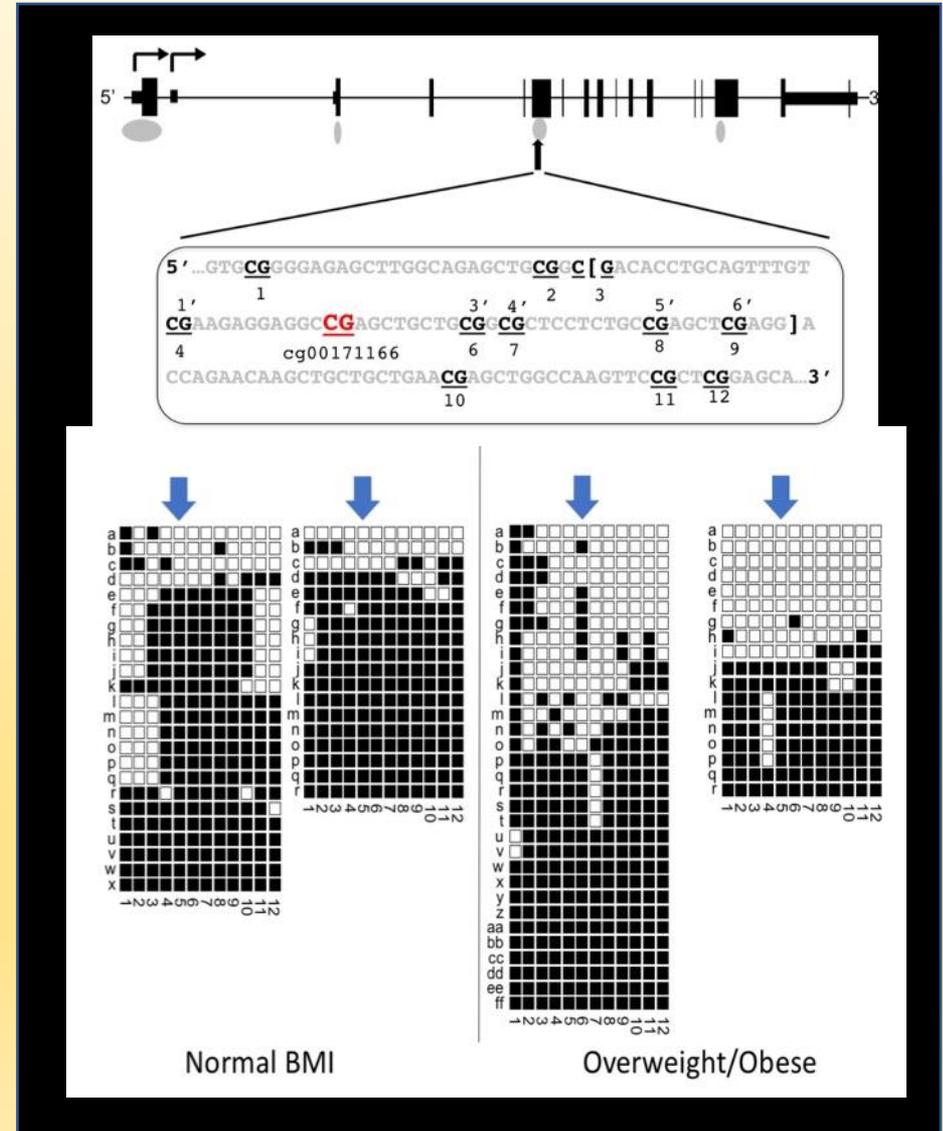
- downstream effector of adiponectin: lowers glucose production

Am J Pathol. 2010 Oct; 177(4): 1936–1945

- differentially methylated in human placenta in association with energy homeostasis

Diabetes. 2016 Dec 16. pii: db160776. [ahead of print]

By 450k, obese had 6% lower methylation

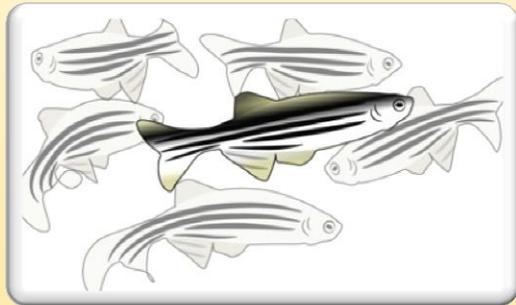




TOBACCO



Tobacco smoke exposure



**Tobacco Smoke Extract
0.1% TPM**



14 days

Sperm
N=6
per group



**Tobacco Smoke Extract
0.2 mg/kg/day**

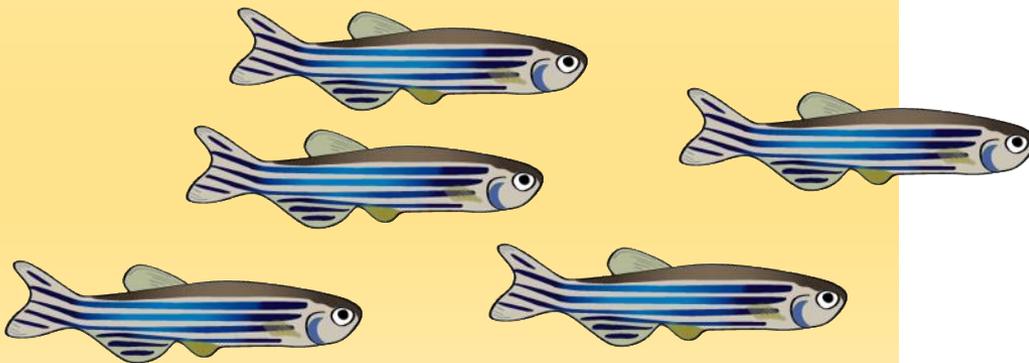


12 days

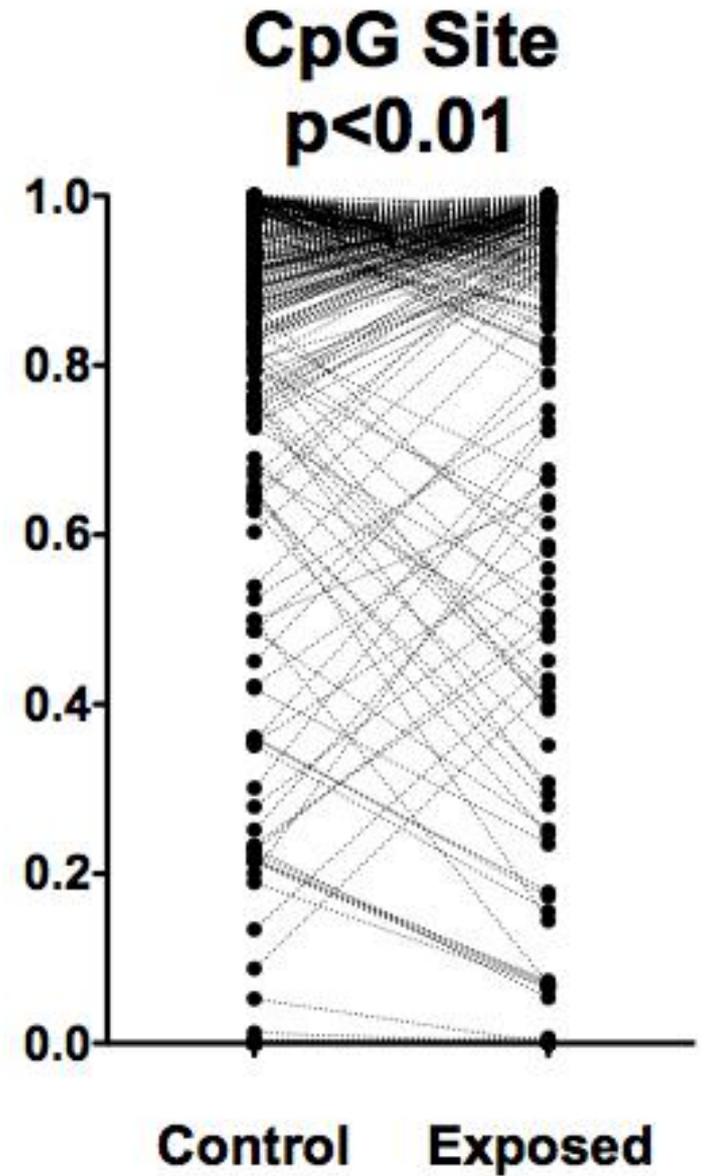
Sperm
N=12
per group

Reduced
Representation
Bisulfite
Sequencing

Paternal zebrafish genome is highly methylated; large effects



Methylation



Sperm, but Not Oocyte, DNA Methylome Is Inherited by Zebrafish Early Embryos

Lan Jiang,^{1,3,7} Jing Zhang,^{1,7} Jing-Jing Wang,^{1,3,7} Lu Wang,^{1,3} Li Zhang,¹ Guoqiang Li,^{1,3} Xiaodan Yang,² Xin Ma,^{1,3} Xin Sun,¹ Jun Cai,¹ Jun Zhang,⁴ Xingxu Huang,⁴ Miao Yu,⁵ Xuegeng Wang,⁶ Feng Liu,² Chung-I Wu,¹ Chuan He,⁵ Bo Zhang,⁶ Weimin Ci,^{1,*} and Jiang Liu^{1,*}

¹CAS Key Laboratory of Genome Sciences and Information, Beijing Institute of Genomics

²State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology Chinese Academy of Sciences, Beijing 100101, China

³University of Chinese Academy of Sciences, Beijing 100049, China

⁴Model Animal Research Center, Nanjing University, Nanjing 210061, China

⁵Department of Chemistry and Institute for Biophysical Dynamics, The University of Chicago, Chicago, IL 60637, USA

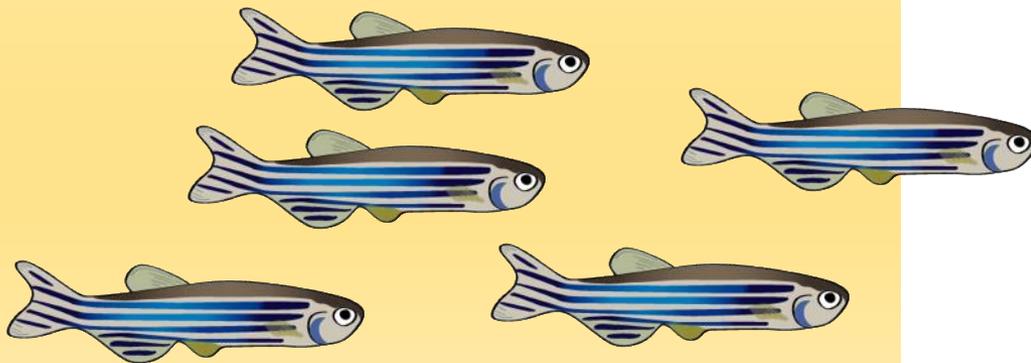
⁶Key Laboratory of Cell Proliferation and Differentiation of Ministry of Education, College of Life Sciences, Peking University, Beijing 100871, China

⁷These authors contributed equally to this work

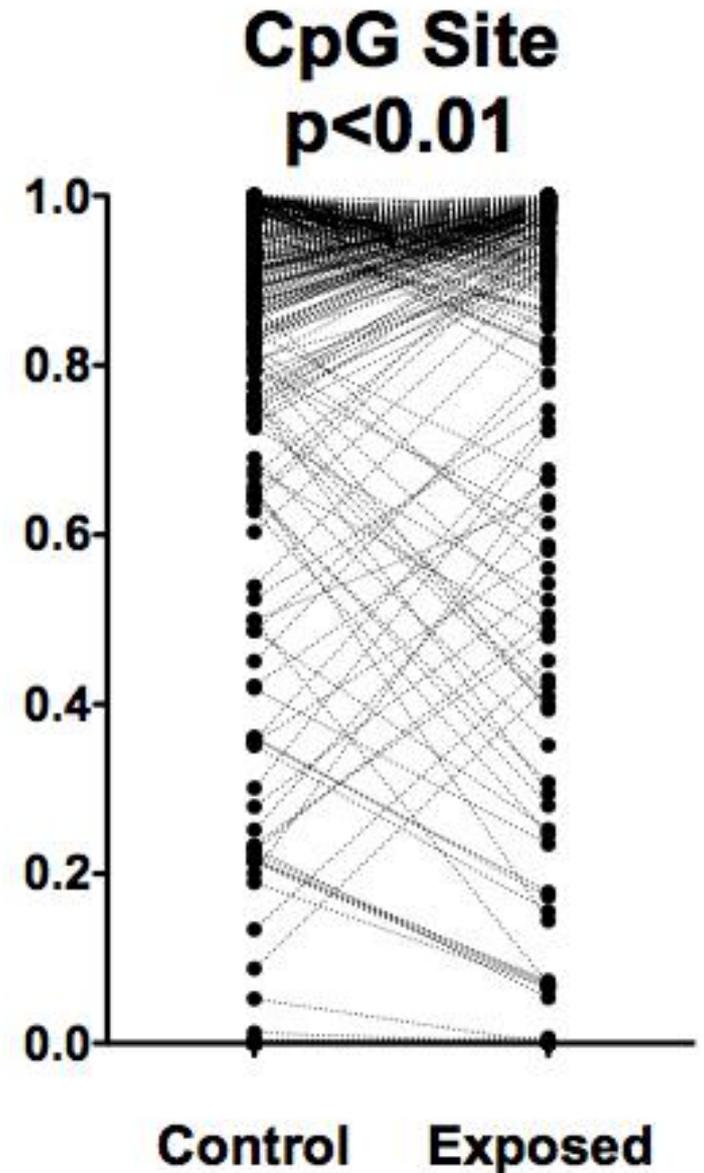
*Correspondence: ciwm@big.ac.cn (W.C.), liuj@big.ac.cn (J.L.)

<http://dx.doi.org/10.1016/j.cell.2013.04.041>

Cell 153, Issue 4, p773–784



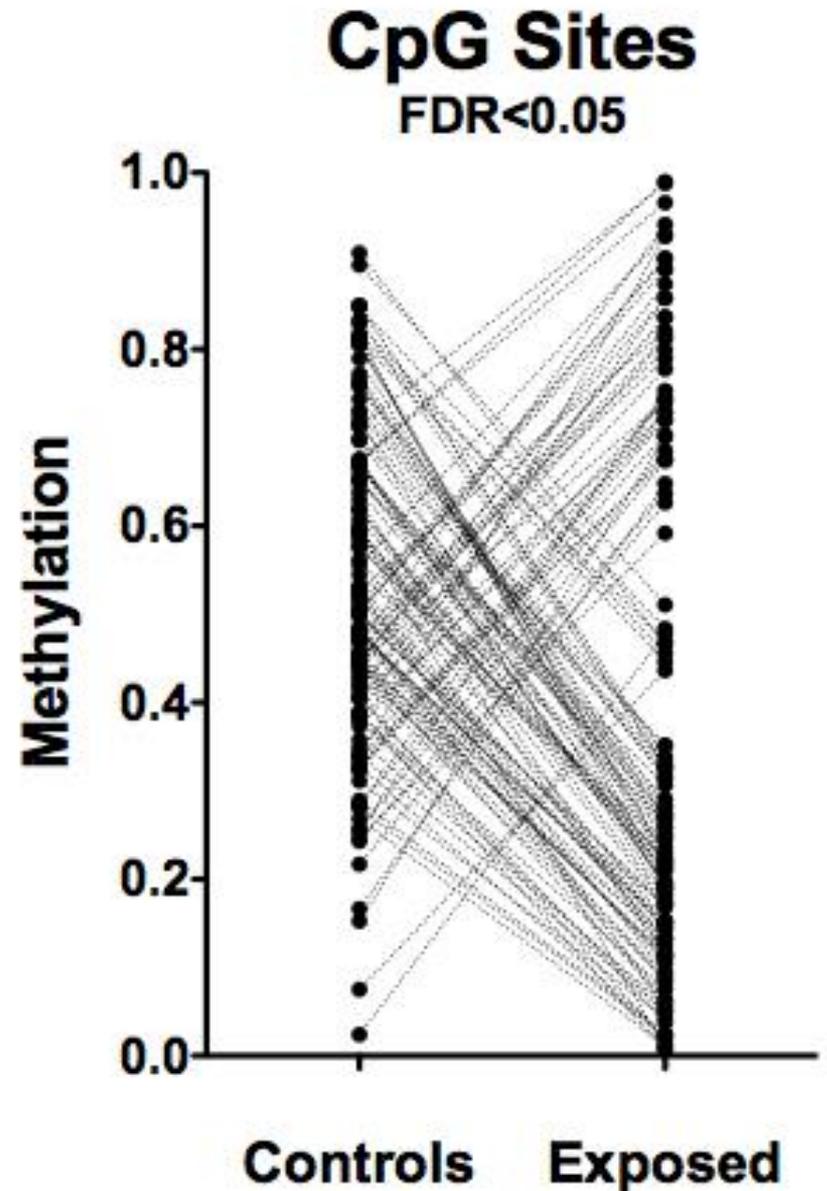
Methylation

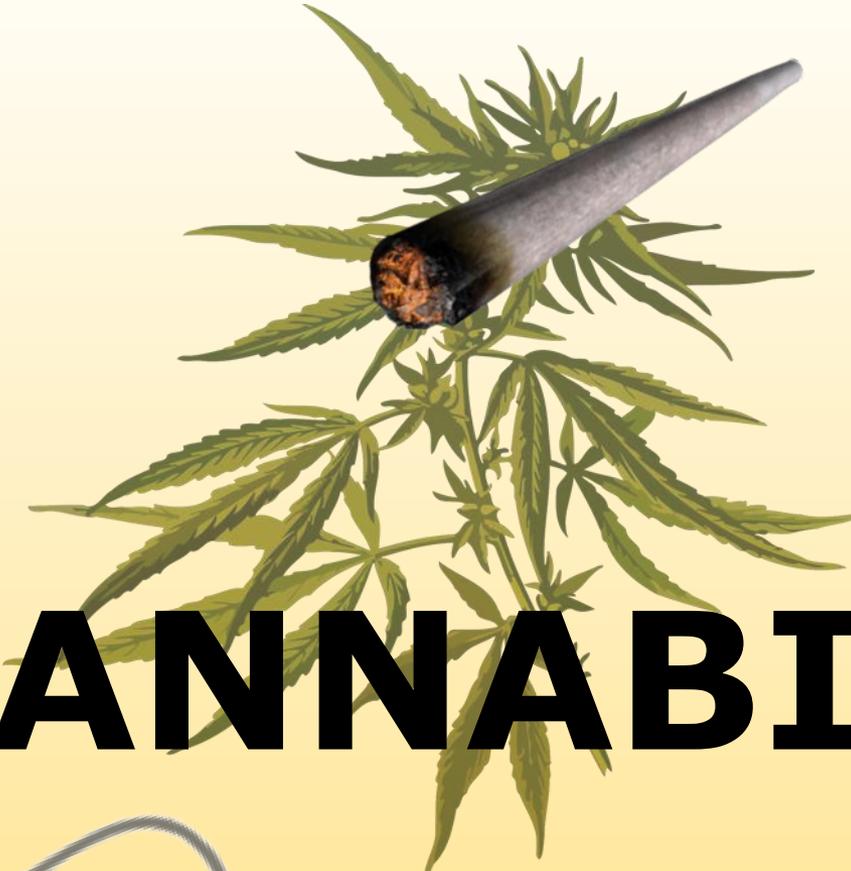


Large magnitude effect sizes



DarkKatze, reddit.com





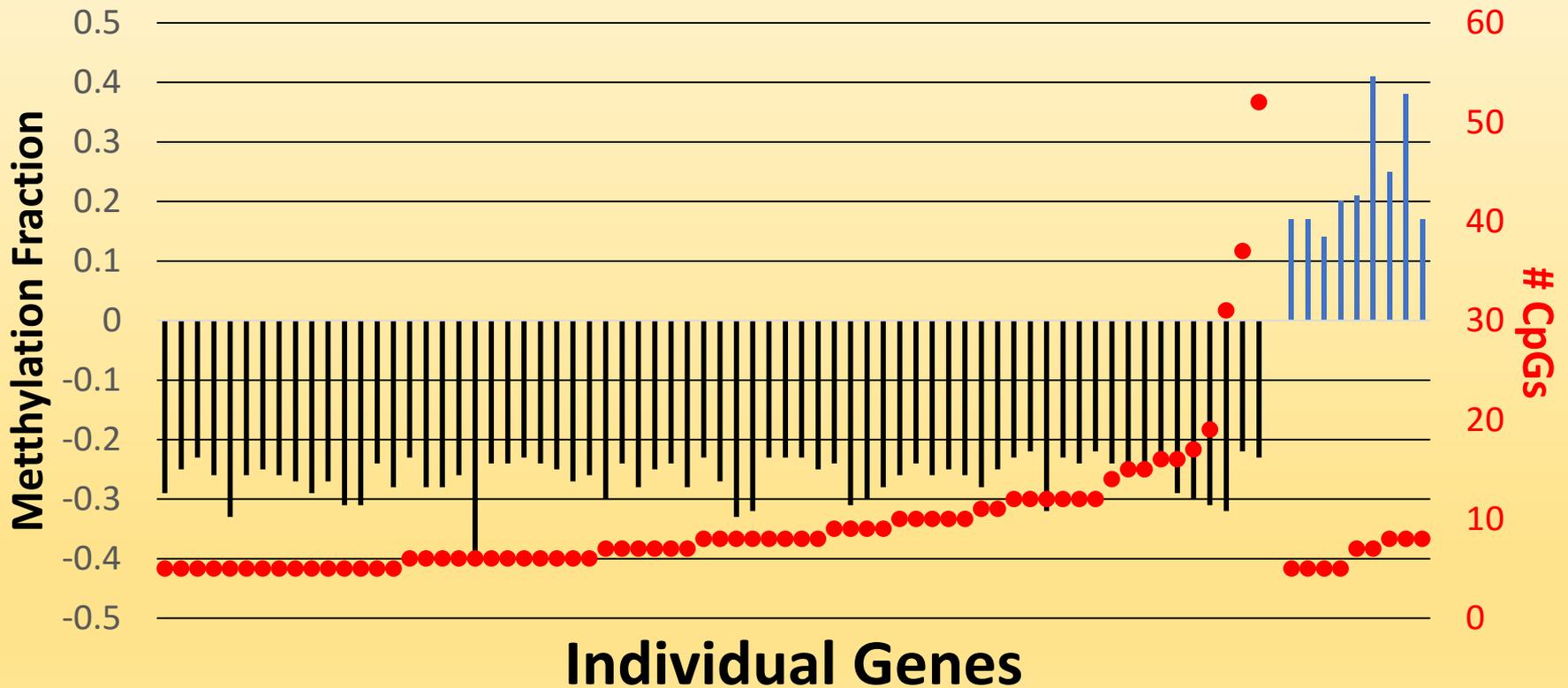
CANNABIS



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

Term	# Genes	P-value	Benjamini
homophilic cell adhesion	16	1.8e-8	3.2e-5
nervous system development	42	8.4e-6	7.3e-3
cell morphogenesis involved in differentiation	16	4.7e-5	2.7e-2
neuron projection morphogenesis	13	6.0e-4	9.9e-2
neurogenesis	24	7.4e-4	1.0e-1

Functions of hypermethylated genes

Term	# Genes	P-value	Benjamini
protein amino acid phosphorylation	45	1.75e-5	3.3e-2
axonogenesis	19	9.9e-5	9.5e-2
axon guidance	13	2.6e-4	1.6e-1
cell morphogenesis involved in neuron differentiation	19	2.7e-4	1.3e-1
neuron projection morphogenesis	19	3.4e-4	1.1e-1

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Emily Liang
Zhiqing
Huang
Amanda Lea
Marty Cauly
Brandon Hall
Emily Burke
Joe Lucas
Ed Levin

Zebra Fish (tobacco)

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Andrey
Massarsky
Amanda Lea
Emily Burke
Joe Lucas
Rich DiGiulio

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Emily Liang
Carole Grenier
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Joe McClernon
Tom Price
Scott Kollins

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