Moving Towards a Scientific Framework for Consideration of Epigenetic Change in Cumulative Risk Assessment

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EPA Workshop 2015

Environmental Susceptibility of the Epigenome

Table 1. Broad environmental epigenetic regulators and references, higher order classifications of toxicants.

	Factor	Observational Epidemiology Citations	Laboratory Toxicology Citations
Toxicant	Heavy metals (Pb, Cd, As, Ni)	(Pilsner et al. 2009) (Wright et al. 2010) (Marsit et al. 2006)	(Bihaqi et al. 2011)
	Air pollution (particulate matter)	(Madrigano et al. 2011) (Tarantini et al. 2009)	(Yauk et al. 2008)
	Persistent organo-pollutants	(Kim et al. 2010) (Rusiecki et al. 2008)	(Zama and Uzumcu 2009)
	Endocrine disrupting chemicals		(Bromer et al. 2010) (Anderson et al. 2012; Guerrero-Bosagna et al. 2008)
Nutrient	One-carbon metabolism	(Ba et al. 2011) (Hoyo et al. 2011) (Hirsch et al. 2008) (Fenech 2001a)	(Mehedint et al. 2010) (McKay et al. 2011)
	Micro-nutrients	(Fenech and Ferguson 2001) (Fenech 2001b)	(Davis and Uthus 2003) (Rowling et al. 2002)
	Caloric restriction	(Tobi et al. 2009)	(Hass et al. 1993)
	Nutraceuticals (EGCG, curcumin, piperine…)	(Yuasa et al. 2009)	(Shi et al. 1994) (Fang et al. 2003)
Pharmaceutical		(Yang et al. 2006)	(Tryndyak et al. 2006)
Lifestyle and Demographics	Smoking	(Breitling et al. 2011) (Joubert et al. 2012)	(Belinsky et al. 2003)
	Socio-economic status	(Borghol et al. 2012) (McGuinness et al. 2012)	
	Stress	(Essex et al. 2013) (Uddin et al. 2010)	(Murgatroyd et al. 2009) (Champagne et al. 2004)

Bakulski & Fallin. **Environmental and Molecular Mutagenesis**

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Environment ~ Epigenotype

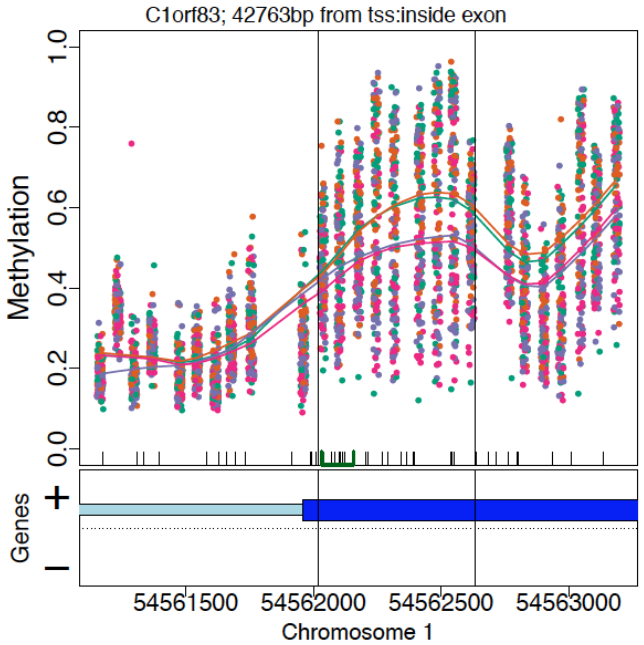
International Journal of Epidemiology, 2015, 1–14
doi: 10.1093/ije/dyv032
Original article

Original article

Prenatal mercury concentration is associated with changes in DNA methylation at *TCEANC2* in newborns

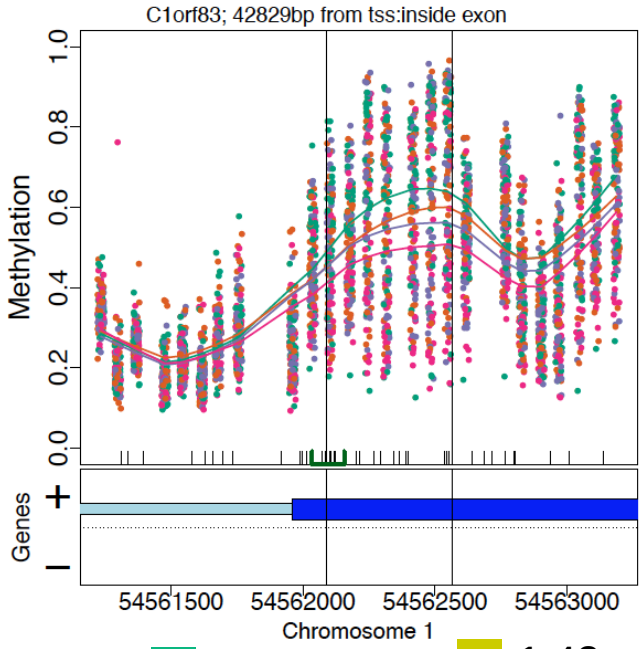
Kelly M Bakulski,[1†] HwaJin Lee,[2†] Jason I Feinberg,[1,2] Ellen M Wells,[3] Shannon Brown,[1] Julie B Herbstman,[1,4] Frank R Witter,[2] Rolf U Halden,[2,5] Kathleen Caldwell,[6] Mary Ellen Mortensen,[6] Andrew E Jaffe,[2,7] John Moye Jr,[8] Laura E Caulfield,[1] Yi Pan,[6] Lynn R Goldman,[1,9‡] Andrew P Feinberg[1,2‡] and M Daniele Fallin[1,2*‡]

MeHg



0.085 - 0.60
0.61 - 0.91
0.93 - 1.6
1.64 – 6.8

Tot Hg



0.23 – 1.02
1.03 – 1.39
1.42 – 1.93
1.97 – 6.3

International Journal of Epidemiology, 2015, 1–14
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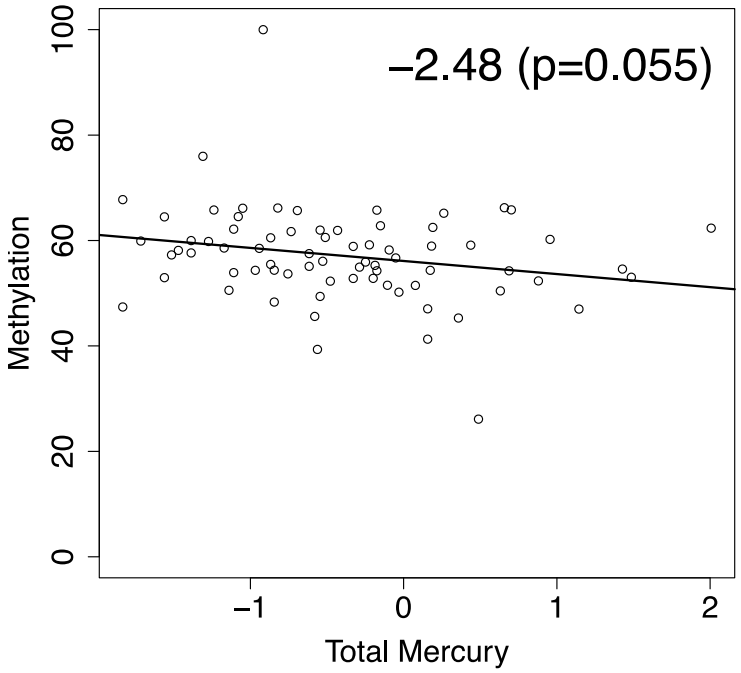
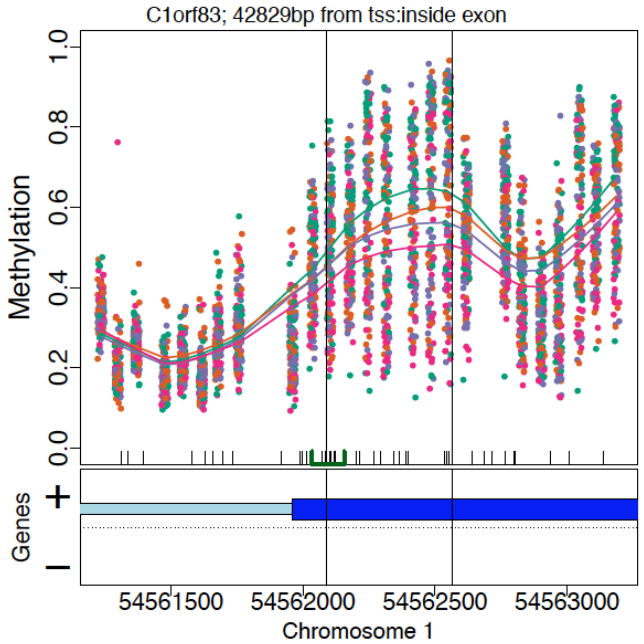
Replication in Independent set of mother-child samples from



Tot Hg



0.23 – 1.02
1.03 – 1.39
1.42 – 1.93
1.97 – 6.3

What does this mean?

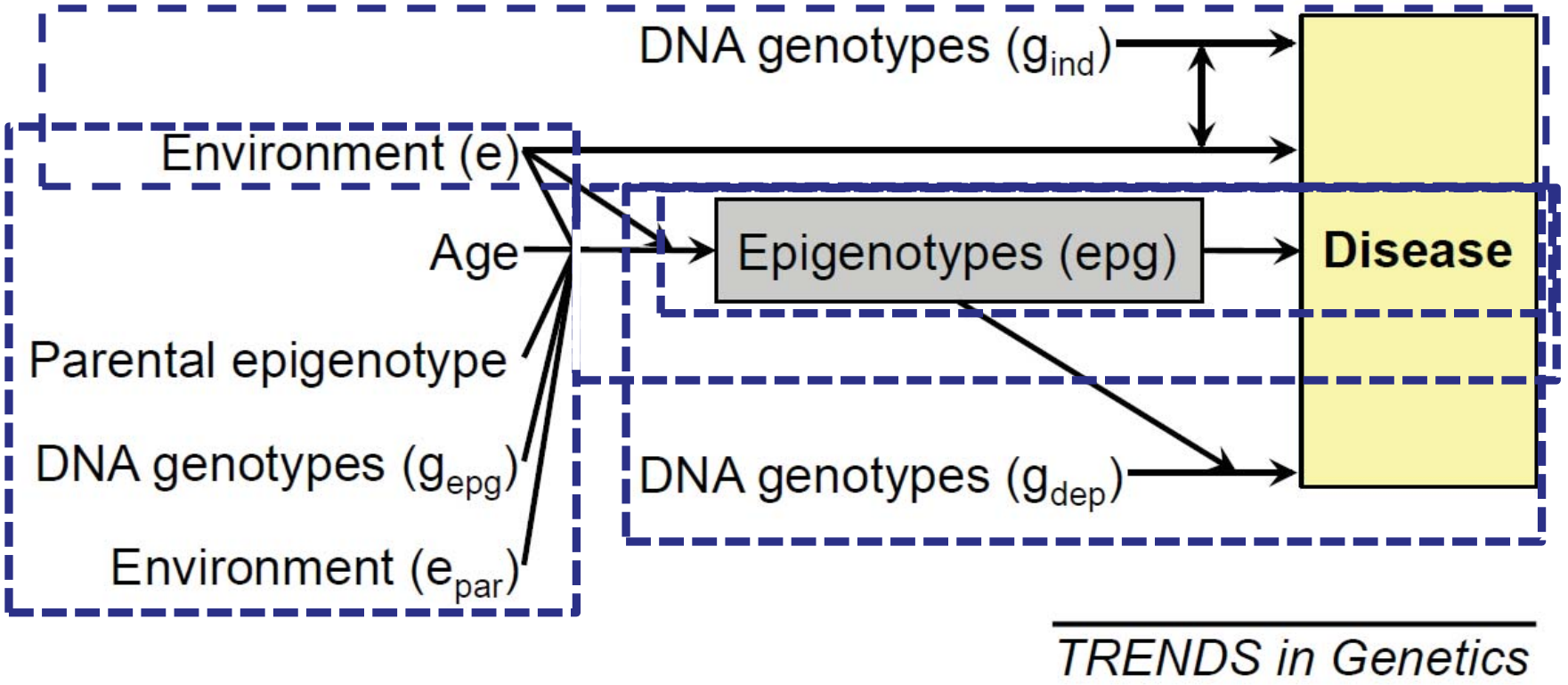
How should we interpret such findings?

How should we go about this moving forward?

Opinion TRENDS in Genetics Vol.20 No.8 August 2004

An integrated epigenetic and genetic approach to common human disease

Hans T. Bjornsson[1,2], M. Daniele Fallin[3] and Andrew P. Feinberg[2]



Utility of Epigenetic Marks for Public Health

Mechanistic:

Mediator of Genetic Risk:

Genotype → Epigenotype → Disease

Mediator of Exposure Risk:

Environment → Epigenotype → Disease

Biomarker:

Biomarker of Exposure:

Environment → Disease

Environment → Epigenotype

Biomarker of Disease:

Disease → Epigenotype

Utility of Epigenetic Marks for Public Health

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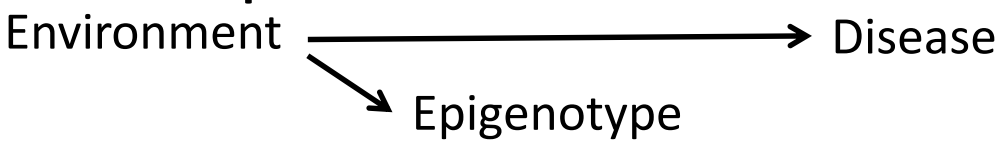
Biomarker:

Biomarker of Exposure:



Utility of Epigenetic Marks for Public Health

Mechanistic:

Mediator of Exposure Risk:

Environment → Epigenotype → Disease

Implications:

- ➢ May provide mechanistic insight into exposure associations
  - Drive research regarding biology of the disease and potential prevention and treatment
- ➢ Epigenetics may be target for intervention
- ❖ Tissue type sampled may be critical

Utility of Epigenetic Marks for Public Health

Mechanistic:

Mediator of Exposure Risk:

Environment → Epigenotype → Disease

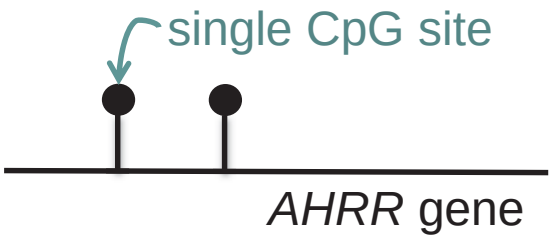
Provide mechanistic insights, Potential targets of intervention, Illuminate GxE interactions

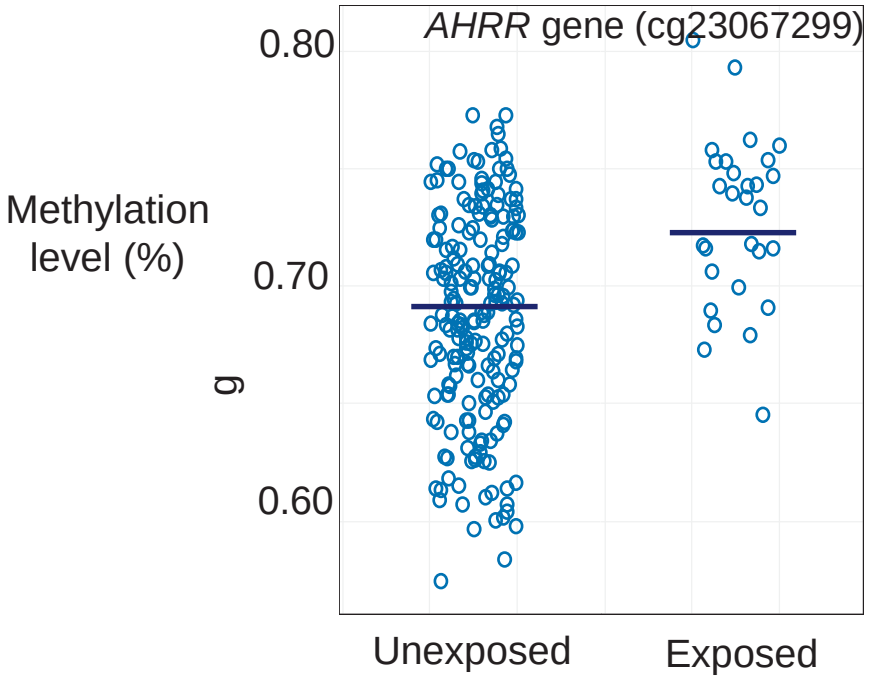
Biomarker:

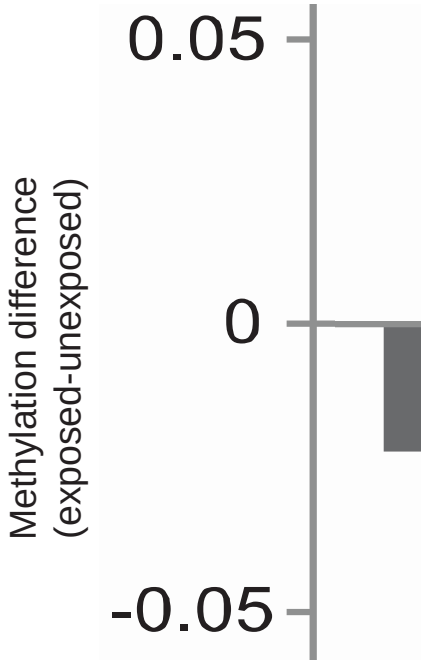
Biomarker of Exposure:

DNAm changes associated with prenatal exposure to smoking

Chris
Ladd-Acosta



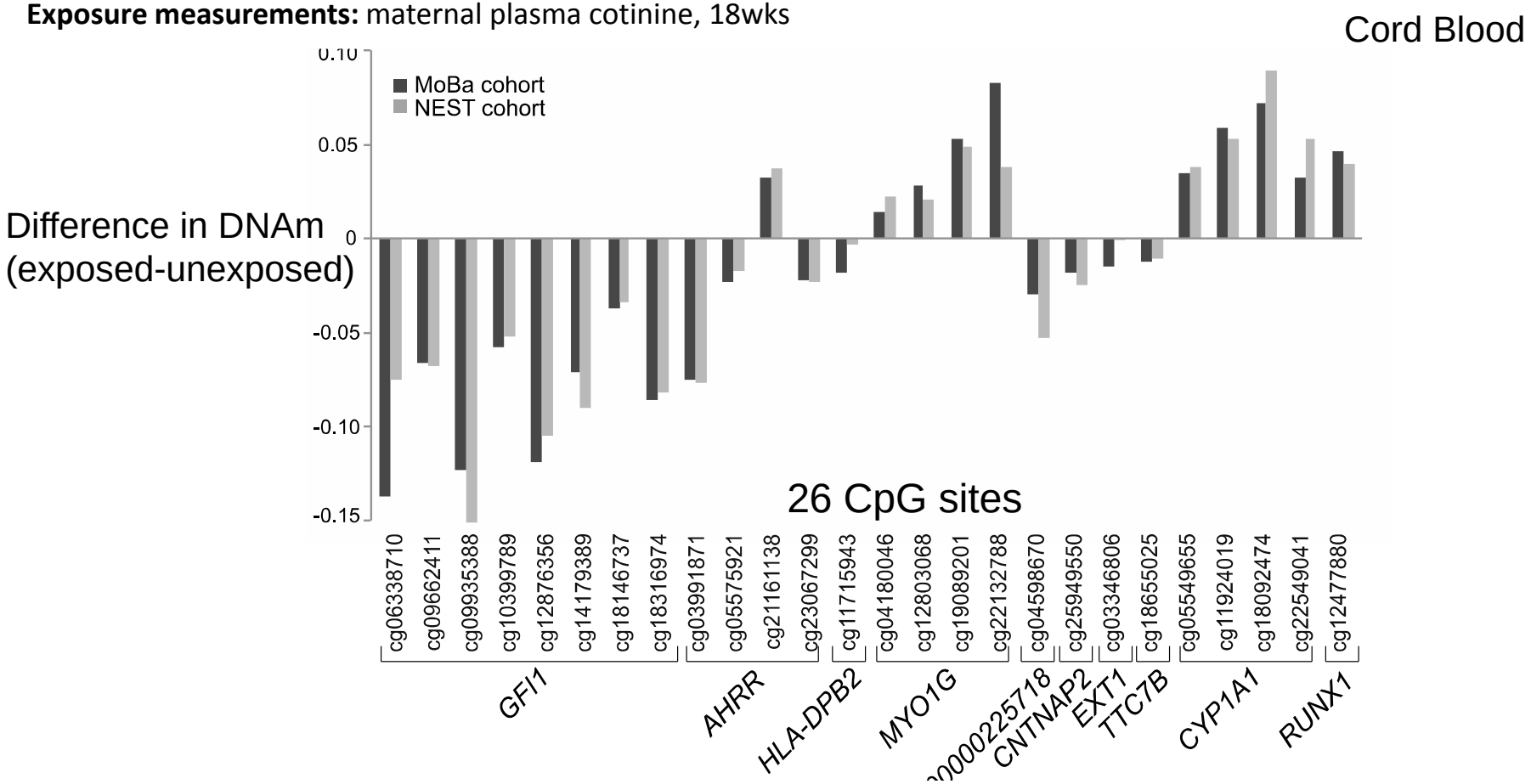




Detectible Cord Blood Methylation Differences By Maternal Smoking in T2

Joubert et al, EHP, 2012

Samples: 1062 newborn cord blood samples (Norwegian Mother and Child Cohort)
Methylation measurements: 485,512 loci (Illumina 450K)
Exposure measurements: maternal plasma cotinine, 18wks

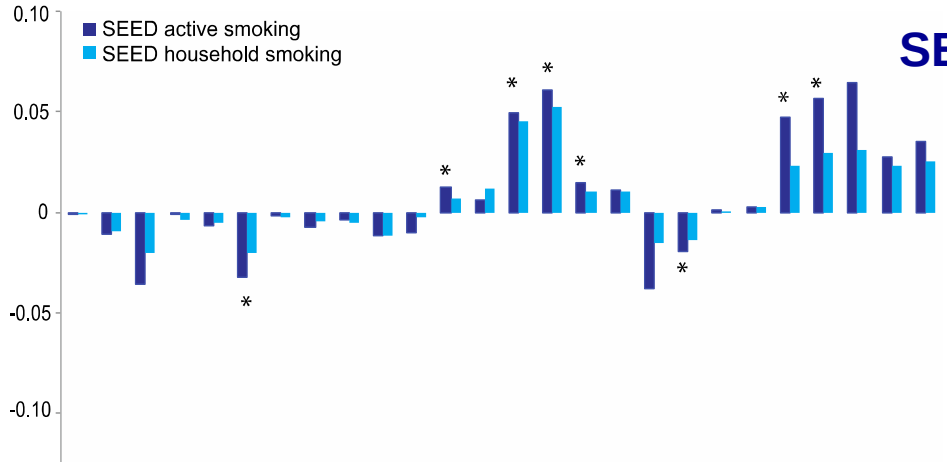


Chris Ladd-Acosta

Epigenetic Signatures of Prenatal Exposure Seen in Newborns and in Young Children

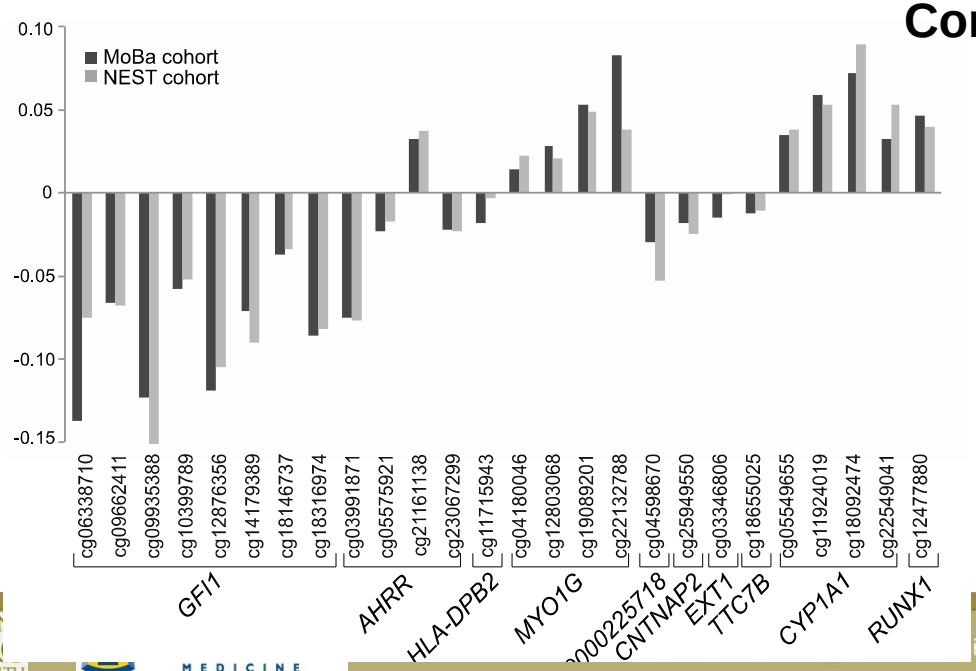
SEED 3-5 year olds

Methylation difference (exposed-unexposed)



Cord blood at birth

Methylation difference (exposed-unexposed)



Utility of Epigenetic Marks for Public Health

Biomarker of Exposure:

Implications:

- Epigenotypes may provide measurable biomarkers of exposure
- May be able to measure past exposure – opens up possibilities for design alternatives or overcoming limitations of particular study designs
- Not causally related, so epigenetics are not the target for intervention, but may be (better) biomarker of cumulative exposure
- Non-target tissue may be useful proxy

Utility of Epigenetic Marks for Public Health

Mechanistic:

Mediator of Genetic Risk:

Genotype → Epigenotype → Disease

Mediator of Exposure Risk:

Environment → Epigenotype → Disease

Provide mechanistic insights, Potential targets of intervention, Illuminate GxE interactions

Biomarker:

Biomarker of Exposure:

Environment → Disease

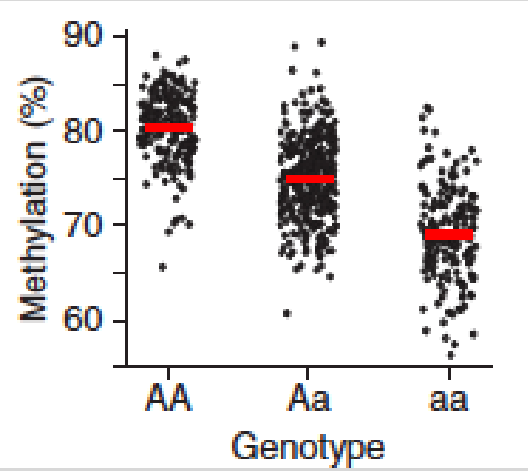
Environment → Epigenotype

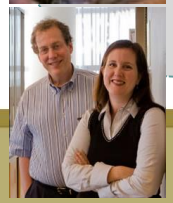
Expand reach of exposure measurement

Why care about Epg Mediation of Genetic Effects for this workshop?

Mediator of Genetic Risk:

Genotype ⟶ Epigenotype ⟶ Disease





Epigenome-wide association data implicate DNA methylation as an intermediary of genetic risk in rheumatoid arthritis

Yun Liu[1,2,12], Martin J Aryee[1,3,12], Leonid Padyukov[4,5,12], M Daniele Fallin[1,6,7,12], Espen Hesselberg[4,5], Arni Runarsson[1,2], Lovisa Reinius[8], Nathalie Acevedo[9], Margaret Taub[1,6], Marcus Ronninger[4,5], Klementy Shchetynsky[4,5], Annika Scheynius[9], Juha Kere[8], Lars Alfredsson[10], Lars Klareskog[4,5], Tomas J Ekström[5,11] & Andrew P Feinberg[1,2,6]

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Mediator of Genetic Risk:

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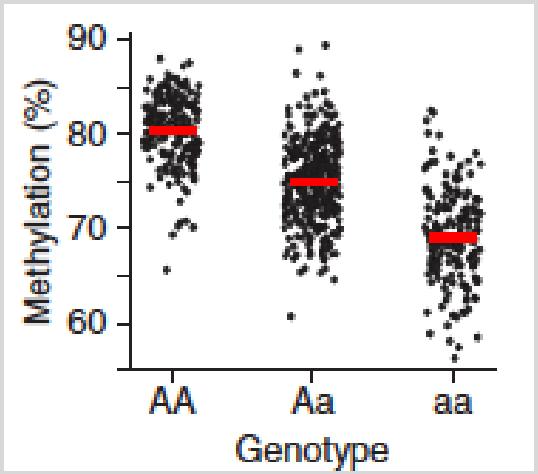

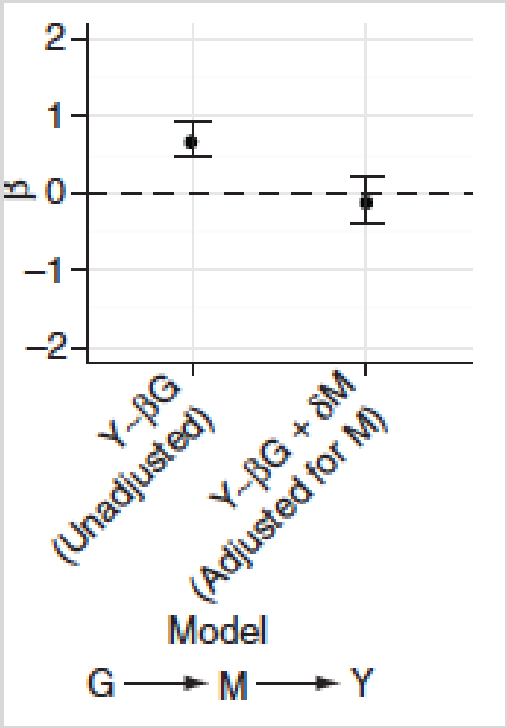

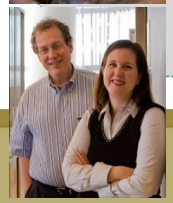

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Mediator of Genetic Risk:

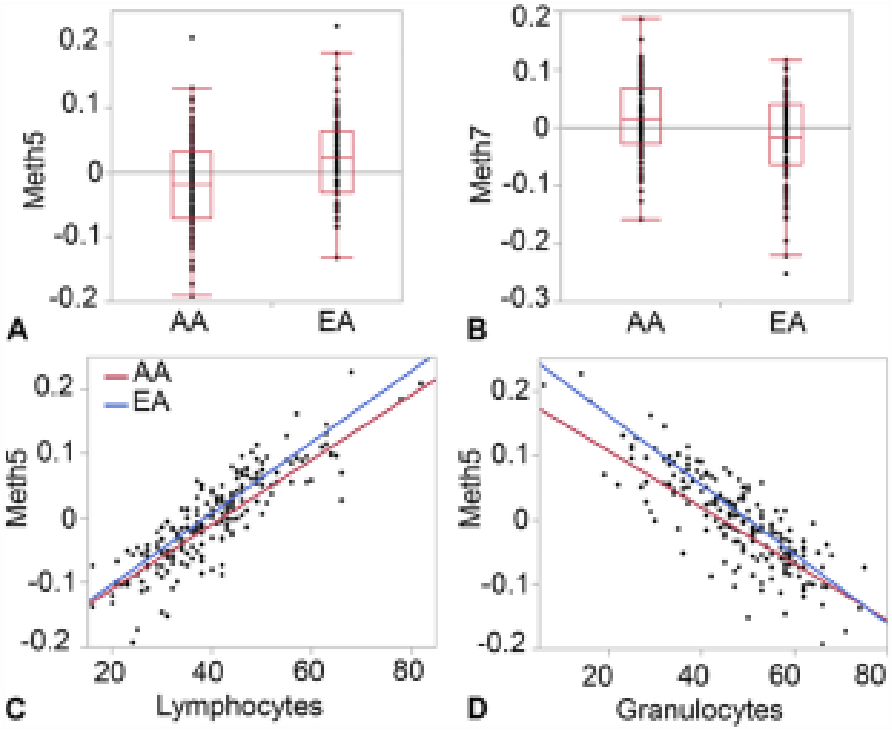
Genotype ⟶ Epigenotype ⟶ Disease

Implications:

- Epigenotype more proximal to disease state – may have higher effect sizes that can drive biological discovery
- Epigenotype is potentially modifiable, genes are (typically) not
- May provide mechanistic insight into genetic associations
  - Drive research regarding biology of the disease and potential prevention and treatment
- **Ancestry can confound exposure associations!**

Genetic Ancestry Can Confound Exposure Associations!

Fig 1. Ancestry-specific methylation modules in CANDLE.



Mozhui K, Smith AK, Tylavsky FA (2015) Ancestry Dependent DNA Methylation and Influence of Maternal Nutrition. PLoS ONE 10(3): e0118466. doi:10.1371/journal.pone.0118466
http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0118466

Utility of Epigenetic Marks for Public Health

Mediator of Exposure Risk:

Environment → Epigenotype → Disease

Provide mechanistic insights, Potential targets of intervention, Illuminate GxE interactions

Biomarker of Exposure:

Environment → Disease

Environment → Epigenotype

Expand reach of exposure measurement

Challenges for Epigenetic Marks of Cumulative Risk

- Tissue availability & specificity (and relevance)
- DNAm Measurement
- Design and timing
- Potential confounding (by age, ancestry, cell type, batch, tissue, etc)
- Load metric
- Statistical approach

	Utility in Public Health	Relevant Tissue
(A) Epigenetics as a MEDIATOR of Exposure Risk: Environment → Epigenotype → Disease	• Identify intervention targets • Illuminate GxE interactions • Provide mechanistic insights into observed associations	• Disease tissue • Surrogate tissue*
(B) Epigenetics as a BIOMARKER of Exposure: Environment → Disease; Environment → Epigenotype	• Expand exposure measurement reach	• Surrogate /disease tissue

*In certain circumstances a surrogate tissue may show the same relationship as diseased tissue

Challenges for Epigenetic Marks of Cumulative Risk

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How Do We Measure DNA Methylation?

An Overview of Scale & Cost

Scale	Name	Type of Method	Amt. DNA Needed	Disadvantages
Global	LUMA	single measurement	500 ng	• does not identify specific loci
Genome-wide, gene-specific	Whole-genome Bisulfite Sequencing	Bisulfite-based	1 ug	• expensive • amount of starting material
Genome-scale, gene-specific	RRBS/SureSelect	Reduced Representation Bisulfite-based	1 ug	• mainly CpG island regions • amount of starting material
	Infinium 450k	Bisulfite-based	500 ng	• genomic coverage
	MeDIP/MBD	Antibody-based	4 ug	• mainly CpG island regions • amount of starting material
	CHARM/HELP	Enzyme-based	3 ug	• amount of starting material
Candidate gene	Bisulfite Pyrosequencing	Bisulfite-based	500 ng	• small number of loci measured

Challenges for Epigenetic Marks of Cumulative Risk

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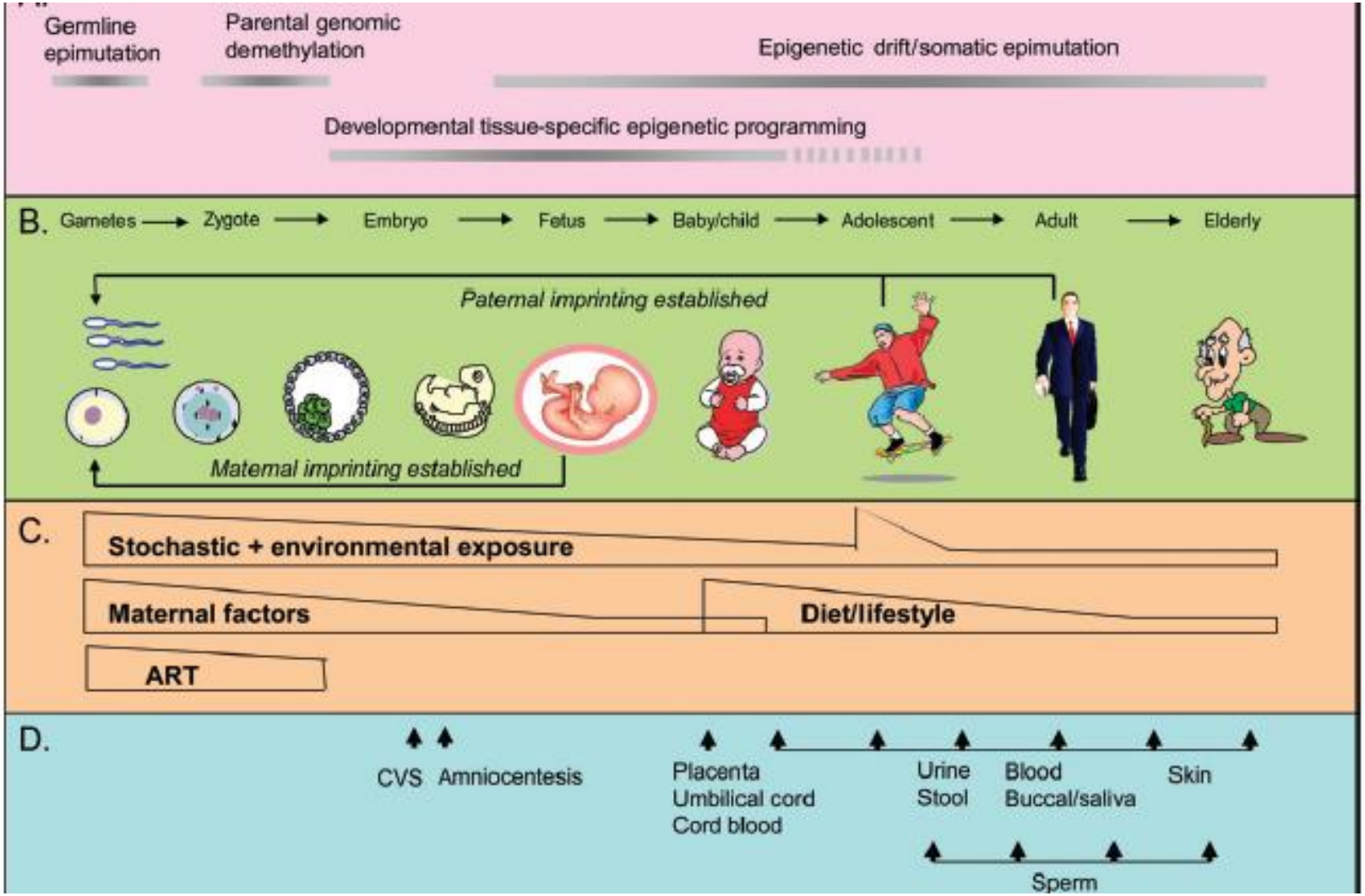

Source: **Foley DL, et al.** 2009. Am J Epidemiol. 169(4):389-400. Prospects for epigenetic epidemiology.

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Cell Heterogeneity: Most tissues are heterogeneous cell mixtures

A set of DNAm sites can distinguish types of cells in blood

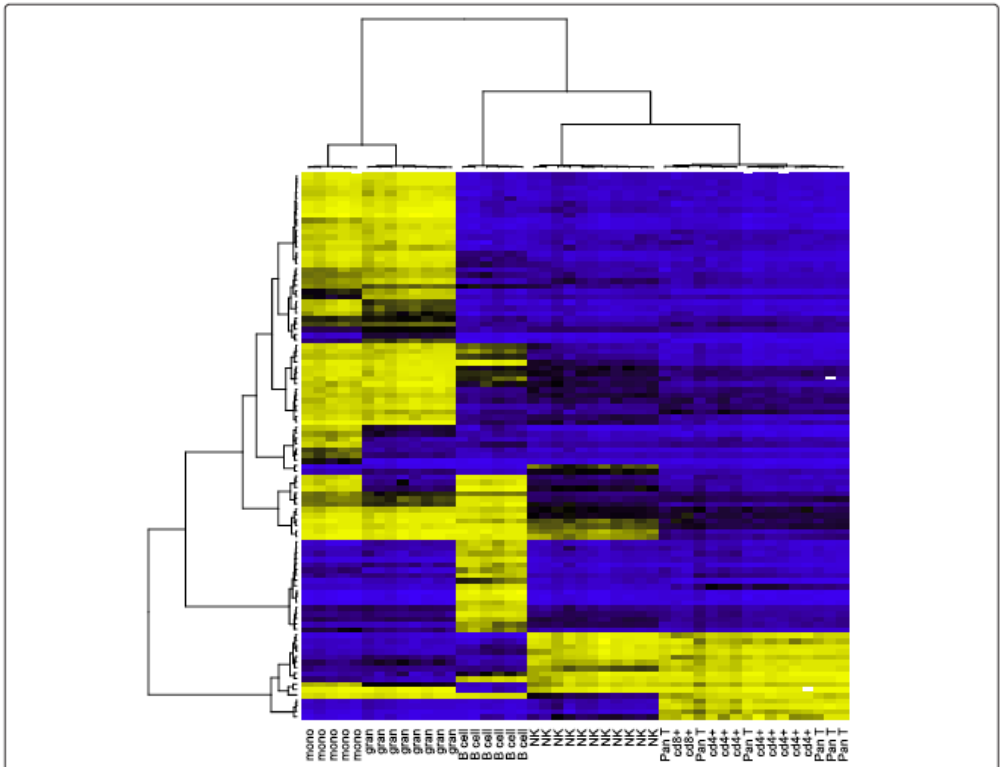


Figure 1 Clustering heatmap for external validation white blood cell data (S_0). Yellow = unmethylated ($Y_{hj} = 0$), black = partially methylated ($Y_{hj} = 0.5$), blue = methylated ($Y_{hj} = 1$).

Estimation and Adjustment for Cell Type in Blood-derived DNA

(1) Houseman estimation – use the DNAm patterns to predict cell type proportions in a mixture (such as whole blood)

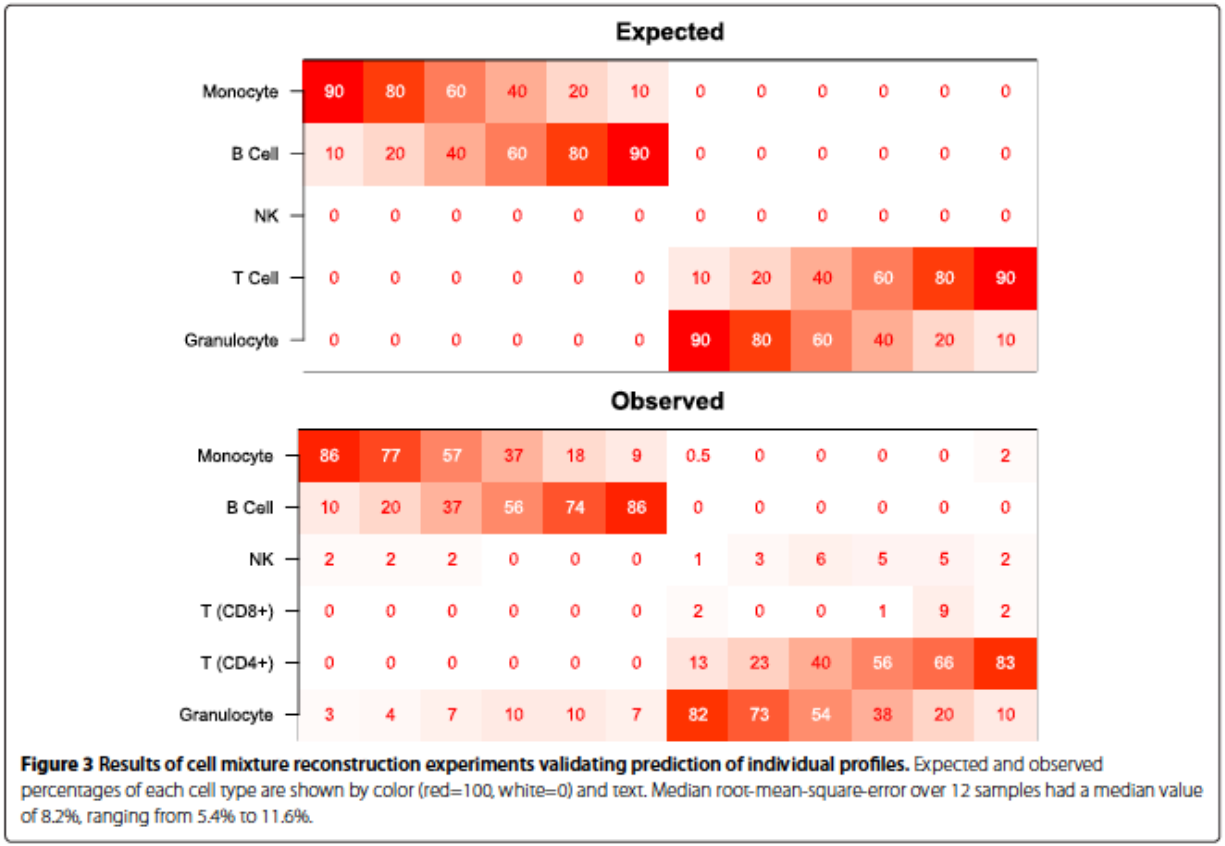
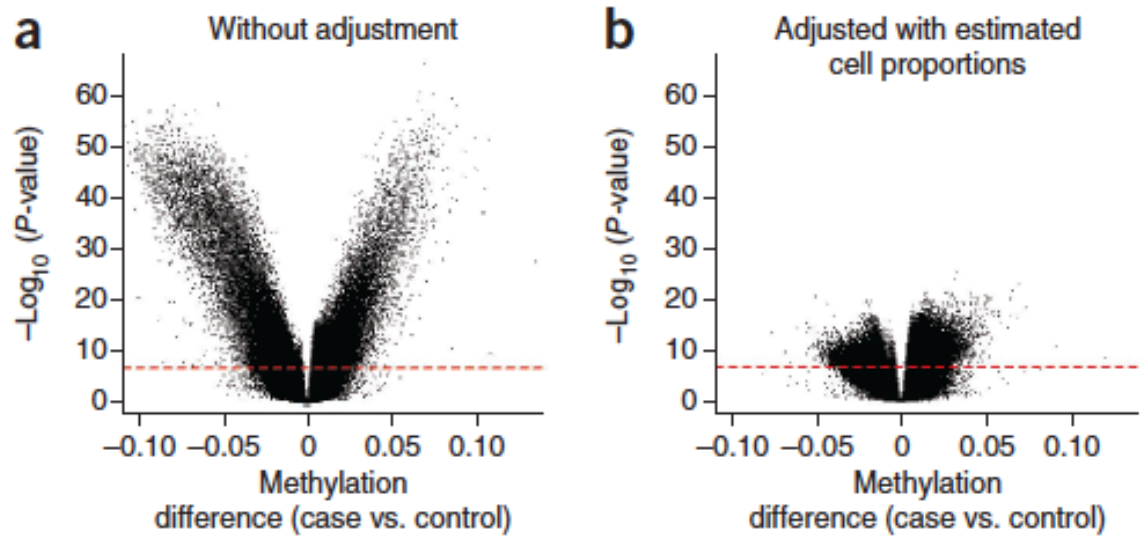


Figure 3 Results of cell mixture reconstruction experiments validating prediction of individual profiles. Expected and observed percentages of each cell type are shown by color (red=100, white=0) and text. Median root-mean-square-error over 12 samples had a median value of 8.2%, ranging from 5.4% to 11.6%.

Estimation and Adjustment for Cell Type in Blood-derived DNA

(1) Houseman estimation
(2) PC on predicted % cell type estimates
(3) Use PCs as adjustment factors



ARTICLES
nature biotechnology

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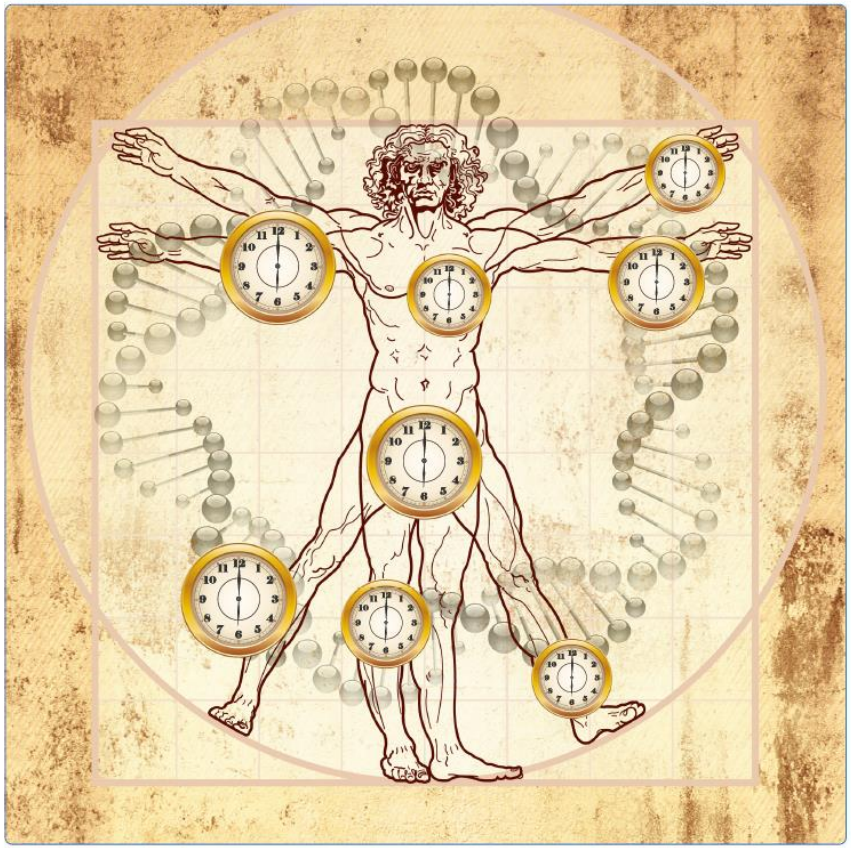
DNAm Signatures of Aging

Genome Biology



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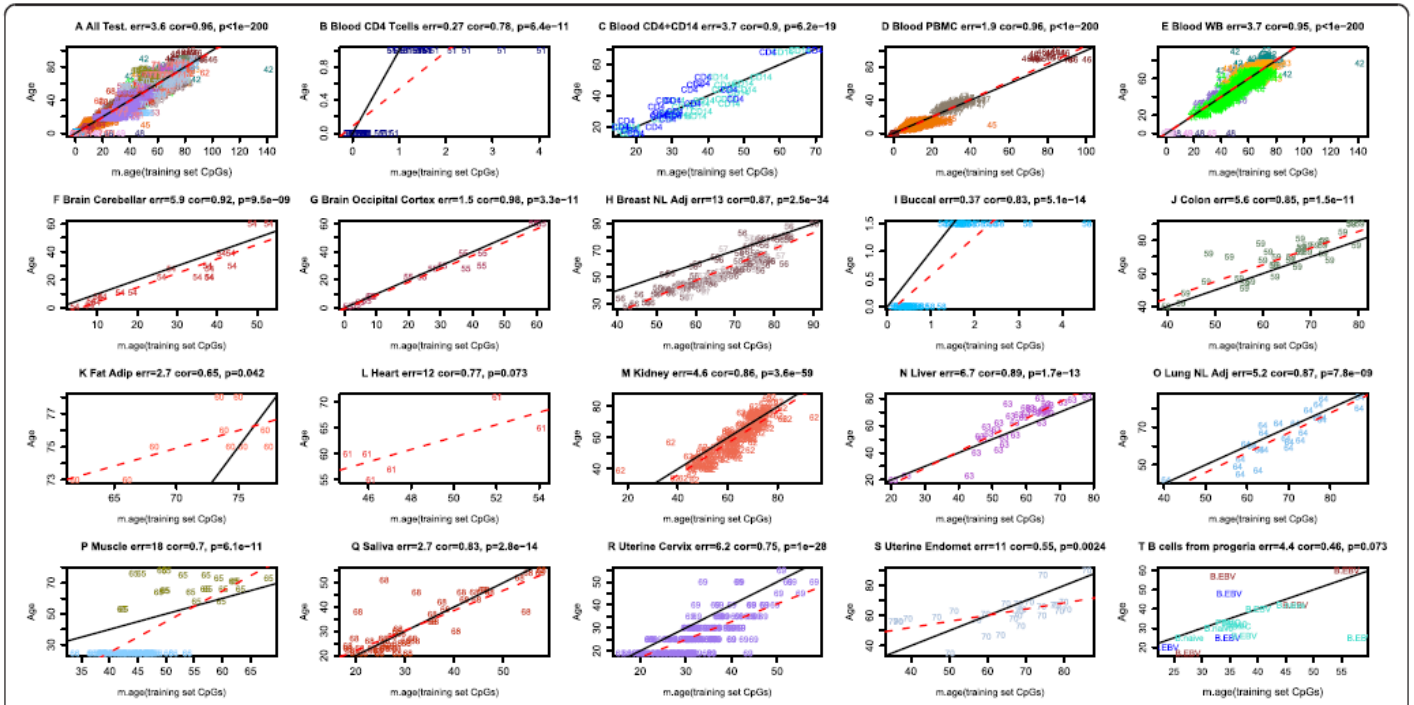


Figure 2 Chronological age (y-axis) versus DNAm age (x-axis) in the test data. (A) Across all test data, the age correlation is 0.96 and the error is 3.6 years. Results for **(B)** CD4 T cells measured at birth (age zero) and at age 1 (cor = 0.78, error = 0.27 years), **(C)** CD4 T cells and CD14 monocytes (cor = 0.90, error = 3.7), **(D)** peripheral blood mononuclear cells (cor = 0.96, error = 1.9), **(E)** whole blood (cor = 0.95, error = 3.7), **(F)** cerebellar samples (cor = 0.92, error = 5.9), **(G)** occipital cortex (cor = 0.98, error = 1.5), **(H)** no
(I) buccal epithelium (cor = 0.83, error = 0.37), **(J)** colon (cor = 0.85, error = 5.6), **(K)** fat adipos
error = 12), **(M)** kidney (cor = 0.86, error = 4.6), **(N)** liver (cor = 0.89, error = 6.7), **(O)** lung (cor = 0.
saliva (cor = 0.83, error = 2.7), **(R)** uterine cervix (cor = 0.75, error = 6.2), **(S)** uterine endometrium
of 10 Epstein Barr Virus transformed B cell, three naive B cell, and three peripheral blood mononu
colored by disease status: brown for Werner progeroid syndrome, blue for Hutchinson-Gilford pr

DNA methylation age of human tissues and cell types

Horvath

BioMed Central

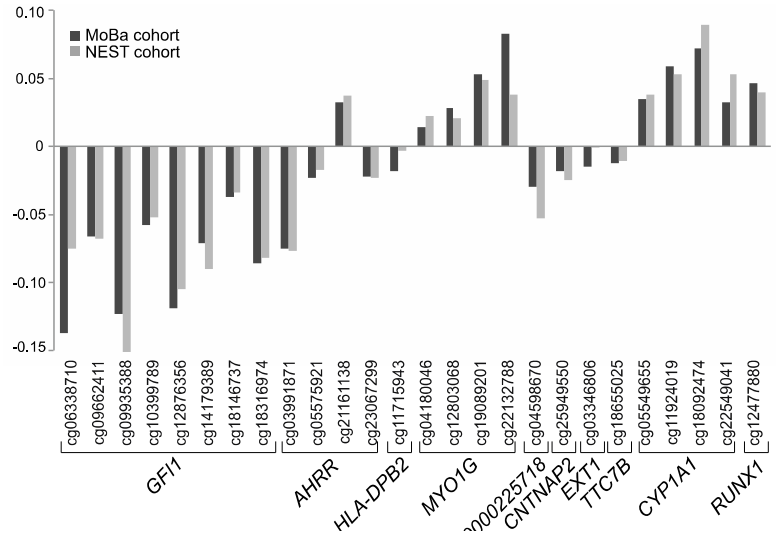
Horvath *Genome Biology* 2013, **14**:R115
http://genomebiology.com/2013/14/10/R115

Challenges for Epigenetic Marks of Cumulative Risk

- ✓ Tissue availability & specificity (and relevance)
- ✓ DNAm Measurement
- ✓ Design and timing
- ✓ Potential confounding (by age, ancestry, cell type, batch, tissue, etc)
- Load metric
- Statistical approach

What metric to use to quantify cumulative exposure epigenetically?

- Individual CpG DNAm levels?
- Unweighted score across CpGs defined by some exposure association threshold?
  - Not as predictive as weighted scores
- Weighted score?
  - Need high-precision weights (large N)



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- ● Statistical approach

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Expand reach of exposure measurement

Our Research Group

- Kelly Benke
- Kelly Bakulski
- Brooke Sheppard
- Jason Feinberg
- Shan Andrews
- Shannon Brown
- Andrew Jaffe (Leiber Institute)
- Weiyan Li

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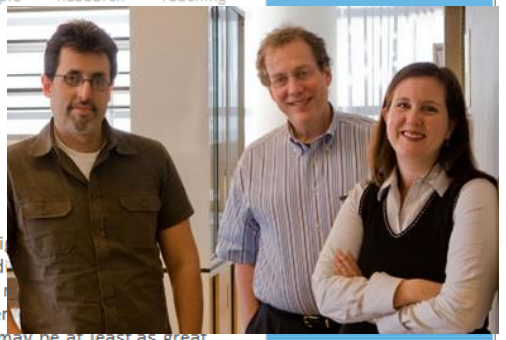
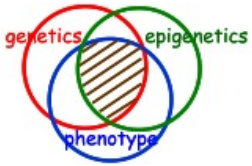
Rafa Irizarry
Sarven Sabunciyan
Hwajin Lee
Michael Multaup
Carolina Montano
Others..





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