Image source: https://epigenie.com/environmental-enrichment-prevents-aging-induced-methylation-changes/

DNA methylation biomarkers of environmental exposures

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The Potential Contribution of Cancer Genomics Information to Community Investigations of Unusual Patterns of Cancer: A Workshop: Session 1

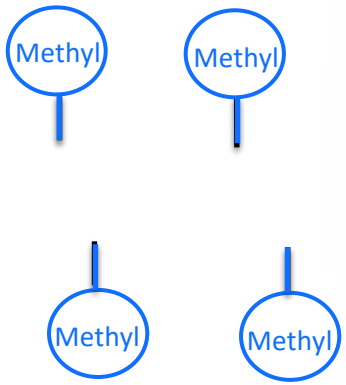
April 13, 2023

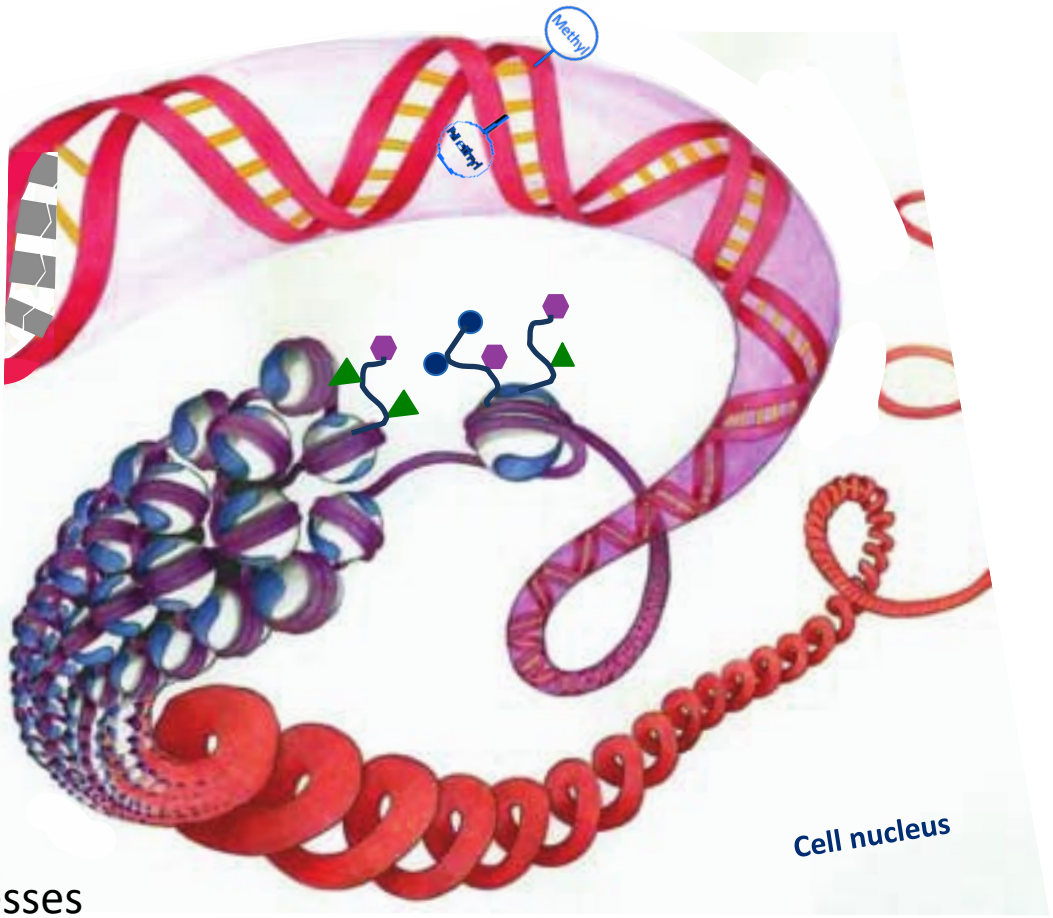
My Disclosures

- Dr. Ladd-Acosta receives consulting fees from the University of Iowa for providing her expertise in autism and epigenetic epidemiology

- Dr. Ladd-Acosta serves as an expert witness for King & Spauling Law

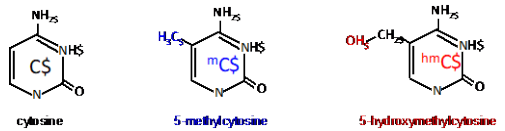
What is DNA methylation?





DNA methylation:

- Occurs at CpGs in humans
- About 28 million CpGs in genome
- Plays a key role in many cell processes
- Can be added/removed without DNA sequence change
- Patterns remembered when a cell divides



Growing evidence for DNA methylation susceptibility to environmental exposures in humans

- Diet
- Metals
- Infection
- Nurture
- Arsenic
- Smoking
- Pollutants
- Childhood SES
- Endocrine disruptors
- Many others……

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 (2014)
https://onlinelibrary.wiley.com/doi/full/10.1002/em.21850

Why are methylation changes associated with exposures important?

1) May inform our understanding of **health/disease MECHANISMS:**

Exposure → Methylation → Health Outcome

"part of the biologic cause"

2) May serve as a **BIOMARKER of Exposure:**

Our focus today!

Exposure → Health Outcome

Exposure → Methylation

"a biologic metric of exposure that may/may not be part of the cause of disease"

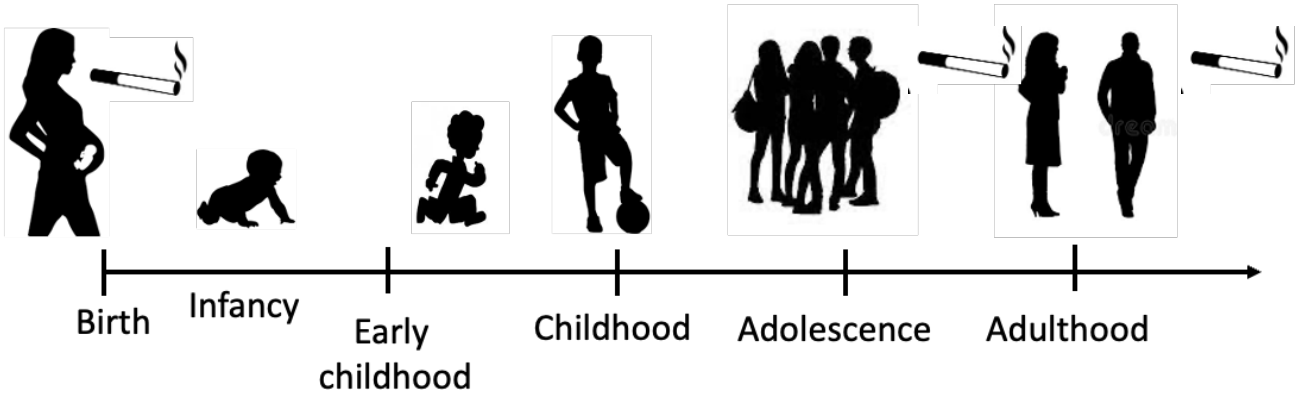
3) May serve as a **BIOMARKER for Health outcomes/disease:**

Methylation → Health outcome → Methylation

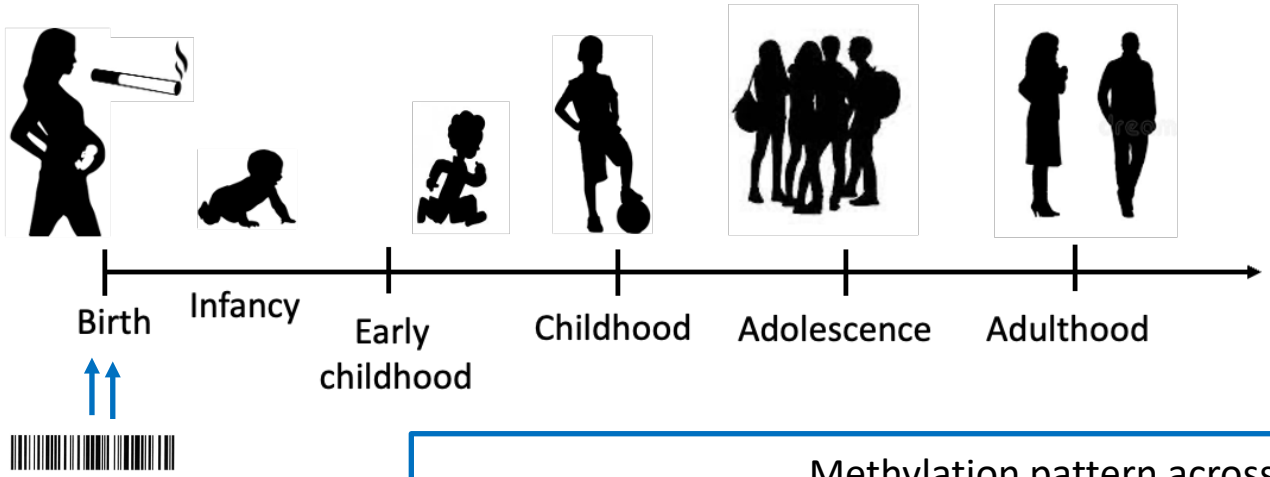
"predict future health" "consequence of disease"

What have we learned so far about DNA methylation biomarkers of exposure?

Proof-of-principle evidence: DNA methylation and smoking

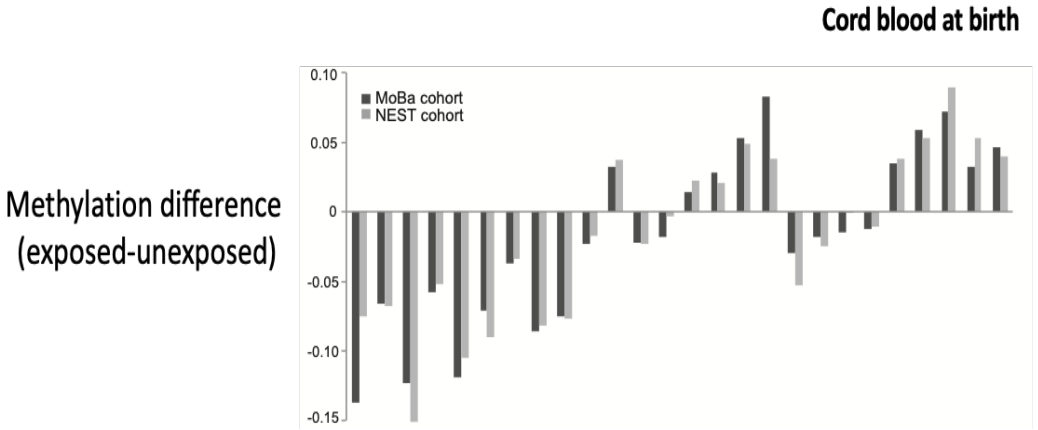


DNA methylation patterns reflect environmental exposures, including prenatal exposures



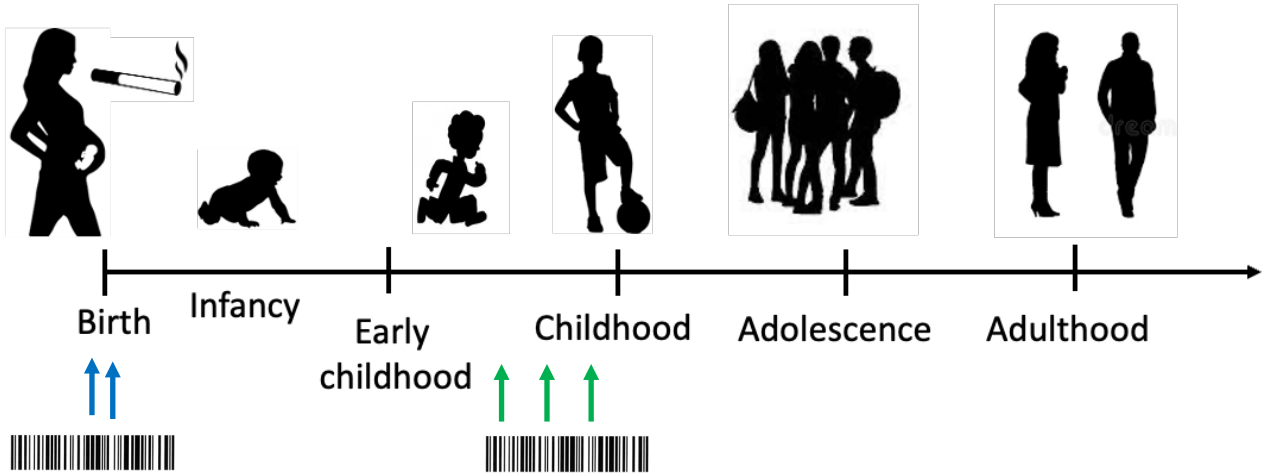
Presence of methylation changes at birth associated with maternal prenatal smoking (cotinine)

Methylation pattern across 26 CpGs



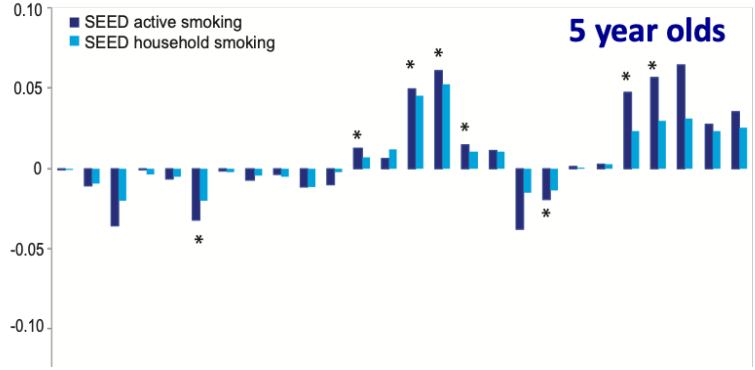
Data re-plotted from Joubert et al. Environ Health Perspect. 2012 Oct;120(10):1425-31.

DNA methylation patterns in childhood samples reflect past historic exposures



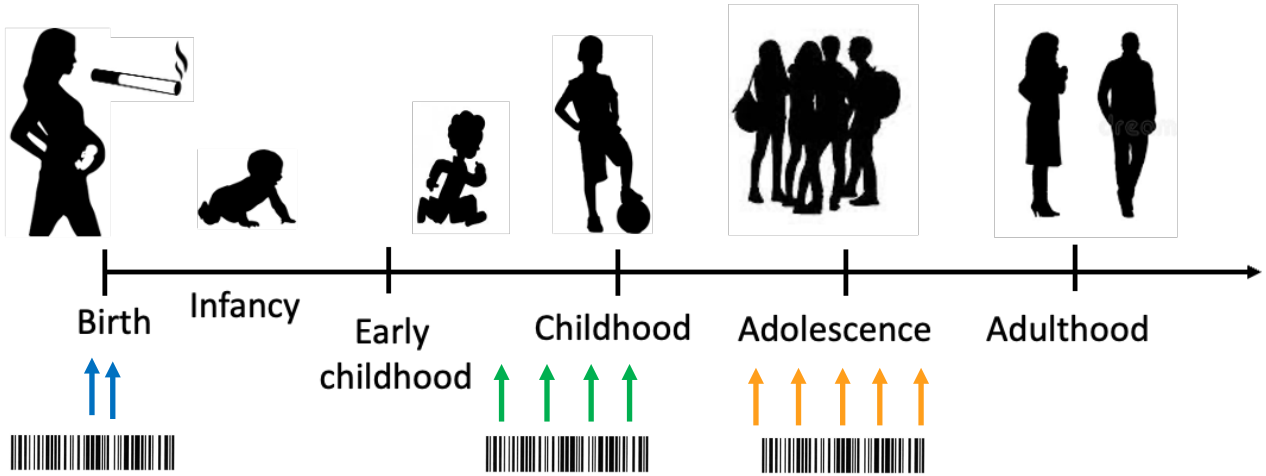
The same methylation patterns are detected in blood from independent set of children **& predict prenatal smoking exposure with 87% accuracy**

Methylation pattern across 26 CpGs



Ladd-Acosta et al. Environ Res. 2016 Jan;144(Pt A):139-148.

DNA methylation patterns in adolescence reflect prenatal smoking exposure

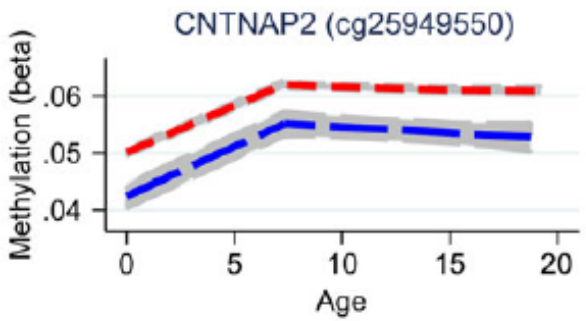


Prenatal smoking methylation patterns are sustained through adolescence , **even after accounting for postnatal (second hand) exposure**

Offspring of non-smoker

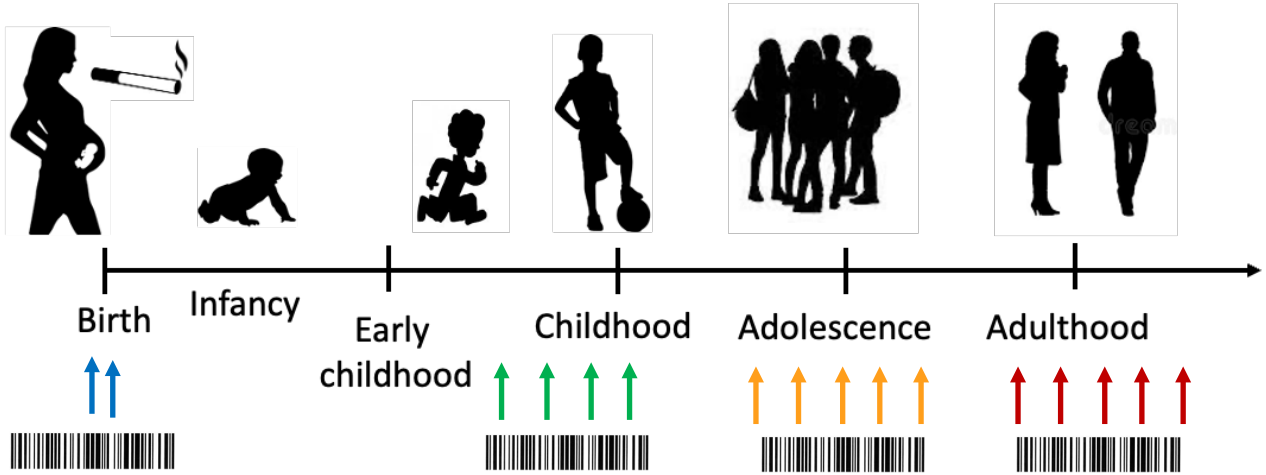
Offspring of sustained smoker

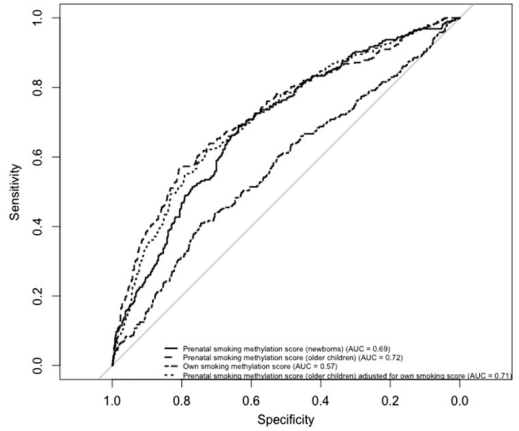
Methylation pattern at 1 exemplar CpG



Richmond RC et al Hum Mol Genet. 2015 Apr 15;24(8):2201-17.

DNA methylation patterns **in adults** reflect prenatal smoking exposure





Prenatal smoking exposure predication accuracy only decreased by 1% when adjusted for own personal smoking history

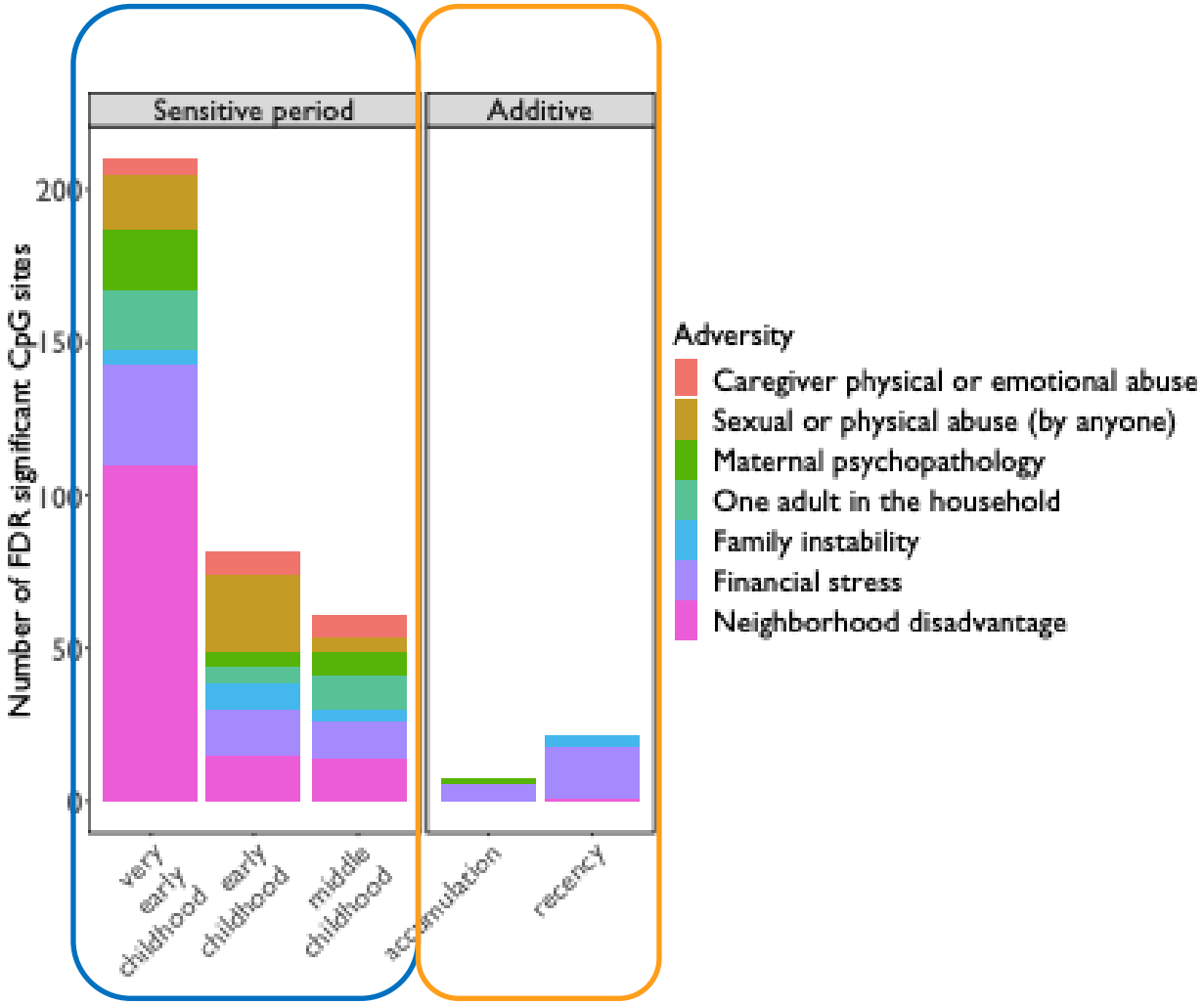
Richmond RC et al Int J Epidemiol. 2018 Aug 1;47(4):1120-1130

Methylation patterns at age 30-53 years predict prenatal smoking exposure, and are **independent of personal smoking history**

Most child methylation patterns of adversity, measured at age 7, reflect specific period of exposure

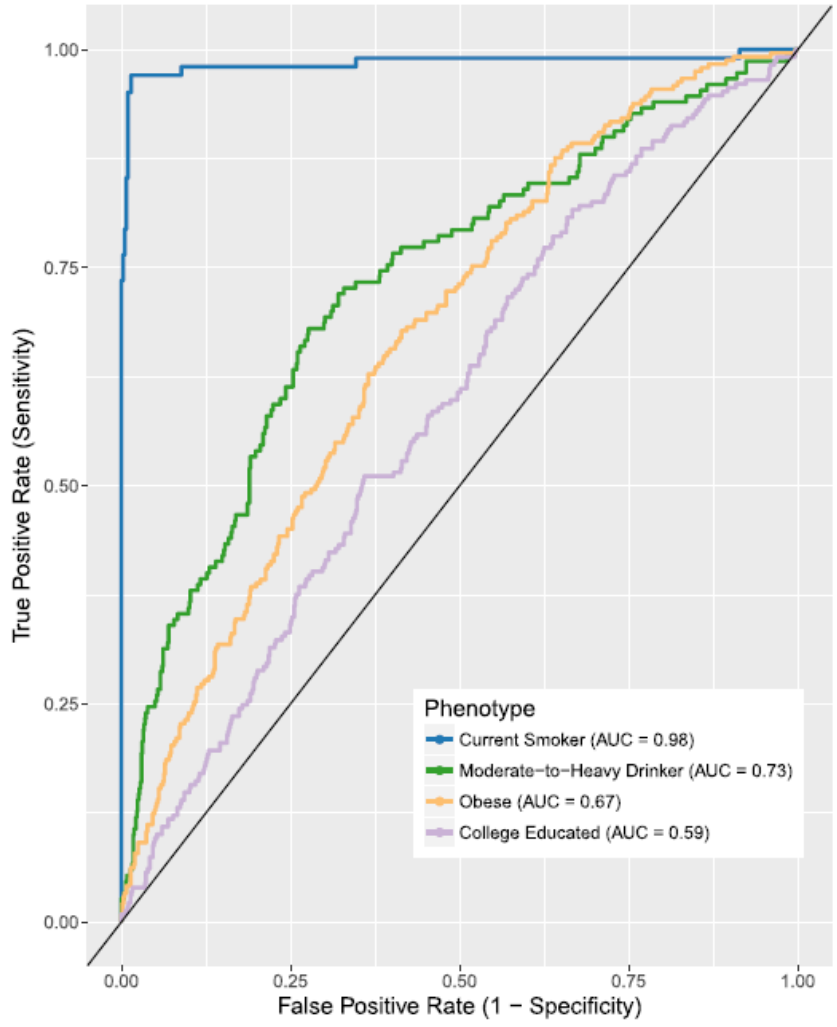
Most 7 year old methylation patterns reflect very early childhood exposure to adversity

Some changes capture cumulative or recent adversity exposures



Dunn EC, et al Biol Psychiatry. 2019 May 15;85(10):838-849.

Other methylation classifiers of exposure are being developed



- **Self-smoking history** epigenetic predictor also available with very good accuracy
- Many others being developed:
  - Prenatal substance use
  - Obesity
  - Prenatal metals
  - Education
  - Others

McCartney DL, et al Genome Biol. 2018 Sep 27;19(1):136

DNA methylation patterns can be used to identify cancer exposure risks and may carry additional "internal dosimeter" info

DNA methylation (score) for smoking history → Incident cancer risk

- ✓ DNA methylation itself can be used to detect exposure risks
- ✓ DNA methylation appears to carry additional/residual risk information

	Hazard Ratio (95% CI)* Highest versus lowest methylation tertile
Lung cancer	
Black	**9.71 (4.61, 20.45)**
White	**10.08 (3.04, 33.41)**
Aerodigestive cancers	
Black	**7.32 (4.03, 13.28)**
White	**4.74 (2.27,9.90)**
Prostate cancer	
Black	0.76 (0.52-1.10)
White	1.12 (0.57-2.22)
Breast cancer	
Black	1.63 (1.04-2.58)
White	0.45 (0.18-1.14)

*all models adjusted for age, diabetes, cell composition, other key covariates

Ladd-Acosta et al, manuscript in preparation

ARIC

In collaboration with the ARIC cancer group: Elizabeth Platz, Corrie Joshu, Miranda Jones, and others

DNA methylation patterns may help with unknown or misreported exposures

Extremely high methylation smoking scores
Lower or no smoking history reported

Cluster of outlier participants

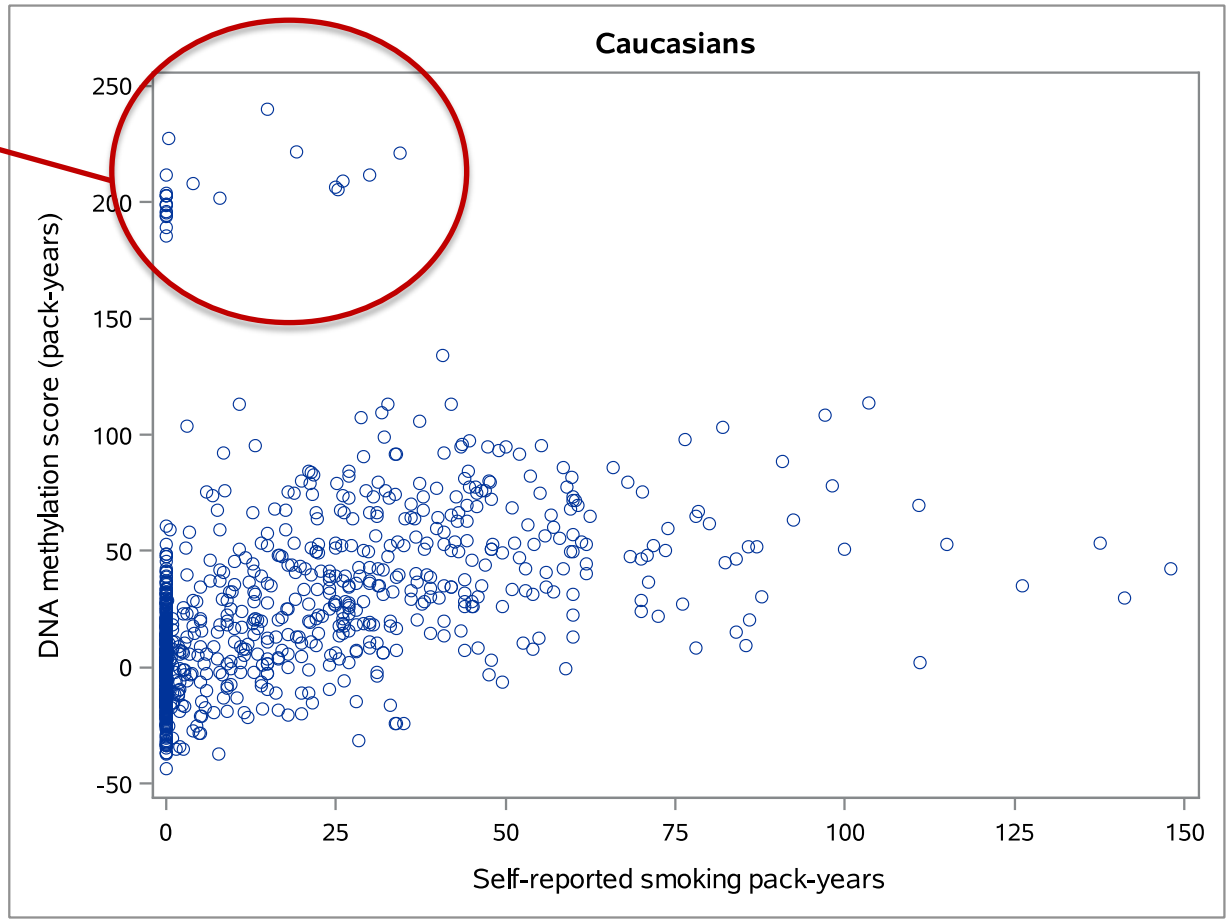
- Less robust data collection?
- Misreported exposure?
- Or…. something else?

Most were from Forsyth Co. NC, a large tobacco/cigarette production area

Photo: Raleigh News and Observer

exposure during tobacco burn offs to clear the land???

employment in tobacco production factories???



https://www.co.forsyth.nc.us/CES/agriculture.aspx
https://en.wikipedia.org/wiki/Winston-Salem,_North_Carolina

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Implications of these findings: Potential for exposure related methylation changes to serve as a useful tool for cancer investigations

1) Environmental risk factor discovery

- Lack of exposure data
- Lack of data for relevant (historic) windows
- Exposure misclassification
- Exposure harmonization
- Cumulative, recent, time window specific
- Internal "dosimeter" of exposures reflecting inter-individual differences in response
- Capture multi-exposures?

May address existing study design challenges and open new possibilities

May provide complementary measures of exposure

Future lines of research needed to realize the full potential:

- Additional exposure studies – particularly for chemical toxicants, across life stages
- Comprehensive genome-wide methylation measures
- Additional method development to built robust and useful predictors
- Reference exposure methylation biomarker databases
- Include diverse participants and subpopulations
- Combine with genetics and/or other biomarkers
- Others….we can discuss!

How else might methylation biomarker tools be useful for cancer investigations: let's think bold and big!

1) Environmental risk factor discovery
   - Lack of exposure data
   - Lack of data for relevant (historic) windows
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May address existing study design challenges and open new study design possibilities

May provide complementary measure of exposure

- Methylation as a "biodetector"

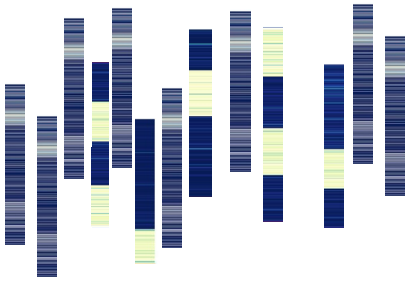
Detect shared exposures among cancer cases/clusters??

How else might methylation biomarker tools be useful for cancer investigations: let's think bold and big!

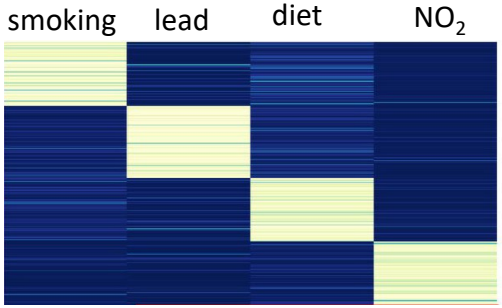
Detect shared exposures among cancer cases/clusters??

1) Input methylation patterns from a sample of individuals

Cancer No cancer



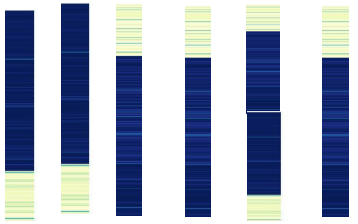
2) Compared to methylation exposure biomarker database



Methylation exposure biomarker reference patterns

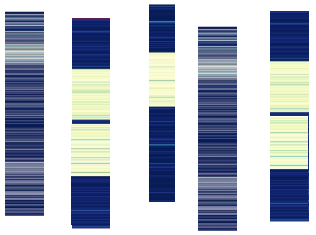
3) Output shared/common exposures within cancer cases compared to control group

Cancer-associated exposure profiles



- Smoking
- Nitrogen dioxide (NO_2)

Non-cancer exposure profiles



- Lead
- Diet

- May improve by combining with genetic variant patterns (McCartney et al Genome Biology 2018)
- Consider designing a custom "exposome array" (improves cost efficiency)

How else might methylation biomarker tools be useful for cancer investigations: let's think bold and big!

1) Environmental risk factor discovery
   - Lack of exposure data
   - Lack of data for relevant (historic) windows
   - Exposure misclassification
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   - Cumulative, recent, time window specific
   - Internal "dosimeter" of exposures reflecting inter-individual differences in response
   - Capture multi-exposures

May address existing study design challenges and open new study design possibilities

May provide complementary measure of exposure

- Methylation "biodetector"

Detect shared exposures among cancer cases/clusters??

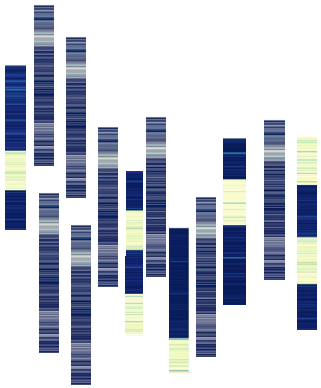
2) Population cancer risk monitoring
   - Methylation "biodetector"

Precision public health (medicine) through identification of possible high risk subpopulations/individuals???
   - informs screening
   - prioritization of intervention resources

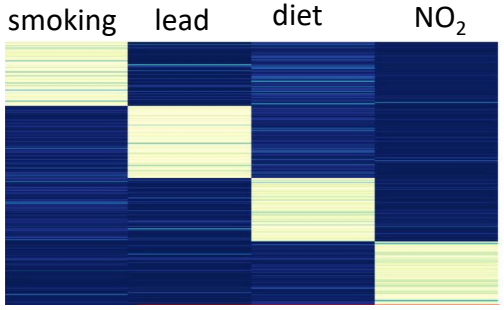
How else might methylation biomarker tools be useful for cancer investigations: let's think bold and big!

Inform precision public health (medicine) through identification of high risk groups???

1) Input methylation patterns from a population

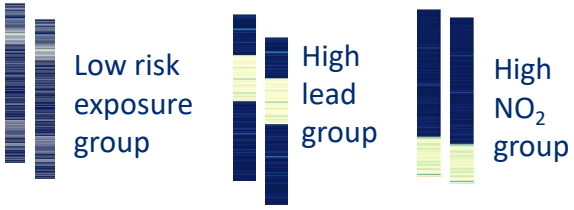


2) Compared to methylation exposure biomarker database



Methylation exposure biomarker reference patterns

3) Cluster individuals to identify groups with similar patterns



4) Output recommendations for subgroups and/or individuals

a) Additional screening

b) Focus intervention resources on this group

Subpopulation 1: geographic cluster

c) Low risk (no further recommendations)

Subpopulation 2

- May improve by combining with genetic variant patterns (McCartney et al Genome Biology 2018)
- Consider designing a custom "exposome array" (improves cost efficiency)

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