

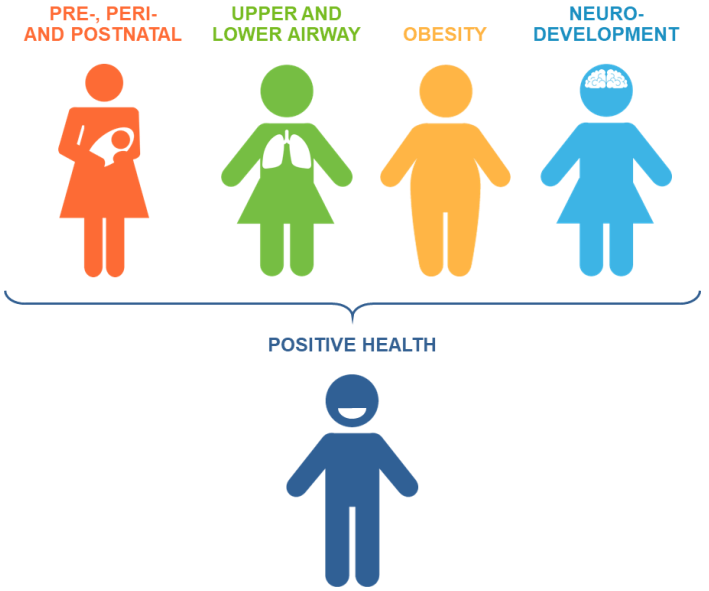
# Prenatal Substance Exposure and DNA Methylation in Children

**Lyndsey Shorey-Kendrick, PhD**
McEvoy Cohort (Award 124)
Division of Neuroscience
Oregon National Primate Research Center
Oregon Health & Science University

ECHO Discovery Series
**June 10, 2026**

# Outline and Goals

- Rationale
  - Prevalence of substance use in pregnancy
  - Effects of substance use on child health
- Limitations of previous studies
- Gaps in knowledge
- What is DNA methylation (DNAm)?
  - How do we use this data and what can we learn from it?
- Previous studies of effects of prenatal substance exposure on DNAm
- Examples in ECHO using DNAm to study effects of prenatal substance exposure
- Opportunities in ECHO to fill knowledge gaps and improve child health



# Rationale

- Substance use during pregnancy remains common

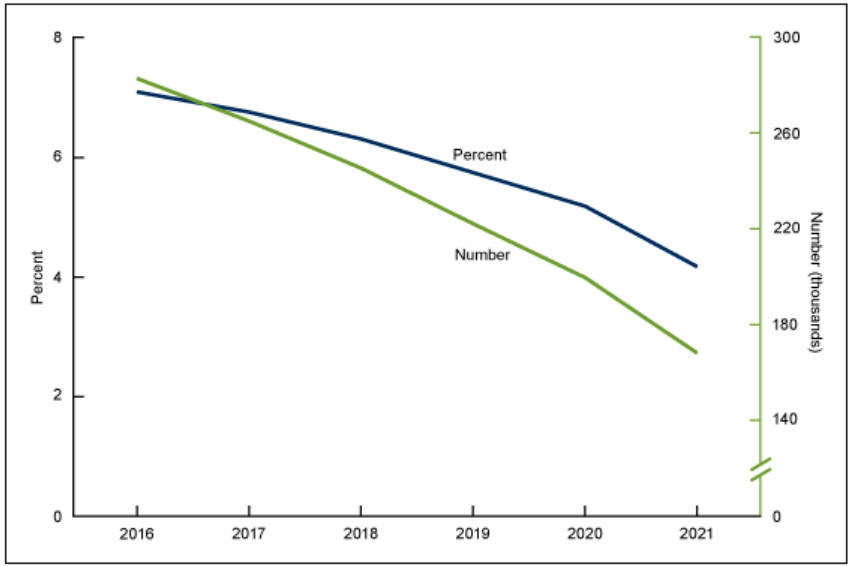
  **Up to 20% report tobacco, alcohol, cannabis or other illicit substance use in pregnancy**

- Prenatal exposures can adversely impact offspring health over the life course
- Molecular signatures can be used to fill **RESEARCH GAPS** to improve health outcomes:
  - Highlight potential underlying mechanisms/pathways
  - Utility as biomarkers to evaluate dose-related associations and identify critical windows of vulnerability
  - Strengthen evidence to inform public policy, health care providers, and pregnant individuals

# Maternal *Smoking* During Pregnancy

- Exposure associated with numerous adverse outcomes in infants and children:
  - Preterm birth
  - Sudden infant death syndrome
  - Lower birth weight
  - Impaired lung development
  - Increased BMI
  - ADHD symptoms, and possible cognitive deficits
- Decreasing prevalence of cigarette smoking during pregnancy, although large variation across the US and increasing use of other nicotine products

**Figure 1. Number and percentage of mothers who smoked cigarettes during pregnancy: United States, 2016–2021**

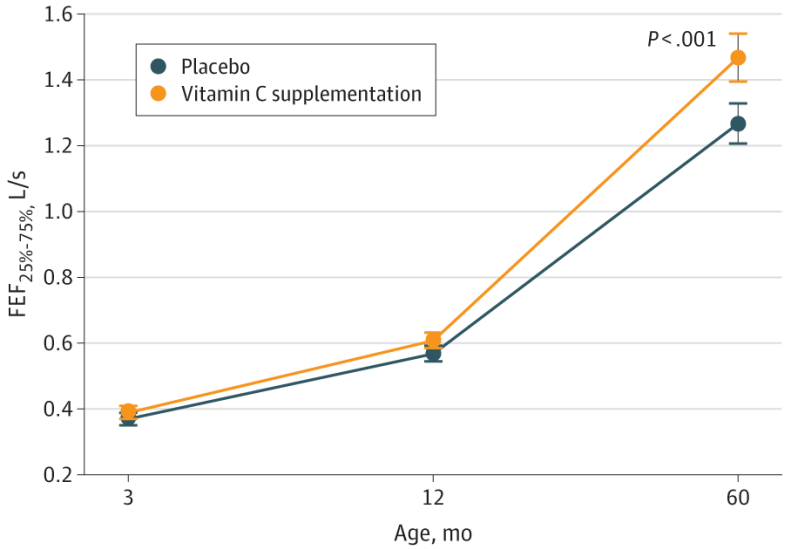



*NCHS Data Brief* ■ No. 458 ■ January 2023

Joyce A. Martin, M.P.H., Michelle J.K. Osterman, M.H.S., and Anne K. Driscoll, Ph.D.

# Vitamin C Supplementation to Pregnant Smokers

- Hypothesis: Vitamin C supplementation during pregnancy will block the adverse effects of maternal smoking on infant pulmonary function
- Methods:
  - Randomized double blinded trial at < 23 weeks gestation
    - Vitamin C (500 mg/day) versus placebo
    - 3 clinical sites
- Lung function - forced expiratory flows measured at 3 and 12 months of age
- Continue to follow these patients, in part through the ECHO funding mechanism

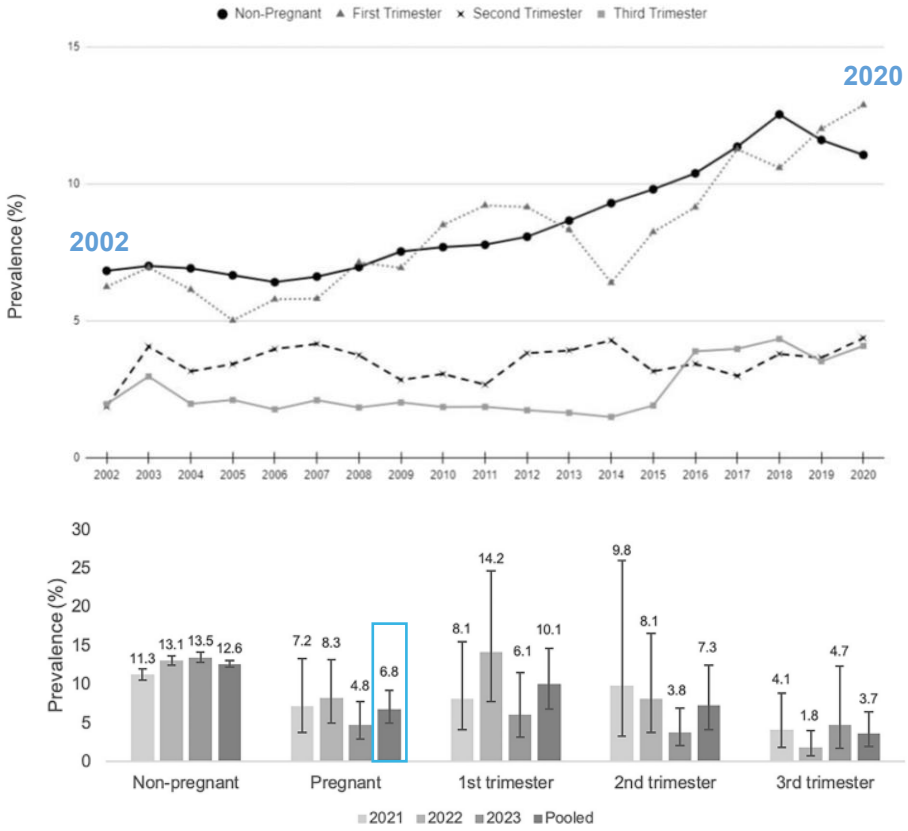



McEvoy CT et al. *JAMA Pediatr*. 2022; McEvoy CT et al. *Eur Resp J*. 2020

# Maternal Cannabis use During Pregnancy

- Increasing with state legalization and social acceptability
- Secondary analyses of data from the National Survey on Drug Use and Health
  - Women aged 12–44 years asked whether they were pregnant and, if pregnant, their trimester of pregnancy
  - Past 30-day cannabis use was the primary outcome (0=no, 1=yes)

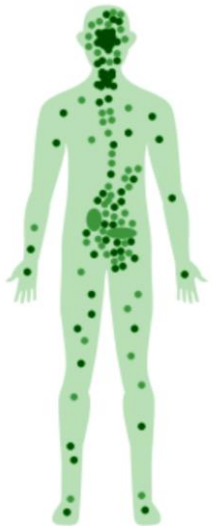
**6.8% of pregnant women reported past month cannabis use 2021-2023**




Hayes et al.,*J Perinatol*. 2023; Grisby et al., *AJPM*. 2025

# Potential Risks of Prenatal Cannabis Exposure





**Fetal/Neonatal**

- Fetal growth restriction and lower birth weight
- Preterm birth
- Increased NICU admissions
- Altered neurodevelopment
- Major structural birth defects

**Childhood**

- Poorer memory, verbal reasoning skills
- Attention/ executive function problems
- Aggressive behavior

**Infancy**

- Decreased ability to self-soothe
- Sleep disturbances

**Adolescence**

- Increased addiction vulnerability
- Increased attention problems

Avalos et al., Birth Defects Res. 2025; Lo et al., JAMA Peds 2025; Lo et al., J Addict Med 2023; Sorkhou et al., Addiction. 2024; Nguyen et al., J Pediatr. 2022; Bassalov et al., Am J Obstet Gynecol. 2024; Keim et al., JAMA Pediatr. 2023

# Other Prenatal Substance Exposure

## Alcohol

- 10.6% of women used during pregnancy
- Increased risk of miscarriage, stillbirth, prematurity, and sudden infant death syndrome, as well as fetal alcohol spectrum disorders (FASD)

Children with FASD are more likely to have:

- Problems eating and sleeping
- Problems with vision and hearing
- Cognitive, behavioral, and academic problems and need special educational services

Pregnancy Risk Assessment Monitoring System (PRAMS) 2022; 2025 NIH Publication No: 21-AA-4101; 2024 National Survey on Drug Use and Health

## Opioids

- 6.6% of women used during pregnancy
- Linked to poor fetal growth, preterm birth, stillbirth, specific birth defects, and neonatal abstinence syndrome (NAS) or neonatal opioid withdrawal syndrome (NOWS)

Newborns with NAS/NOWS are more likely to have:

- Problems eating and sleeping
- High pitched cry, nervous system dysregulation

Pregnancy Risk Assessment Monitoring System (PRAMS) 2019; Vital Signs: Prescription Opioid Pain Reliever Use During Pregnancy — 34 U.S. Jurisdictions, 2019

# Limitations of Prior Studies

- Often limited to self-report and recall bias
  - Lack of information on dose, frequency, route of exposure
- Substance use during pregnancy is often confounded with:
  - Other substance use
  - Genetic risk
  - Environmental exposures
  - Preexisting conditions
- Limited sample size and generalizability
- Few large studies have detailed exposure data, detailed or longitudinal outcome data, and biospecimens to identify molecular mechanisms



# Gaps in Knowledge

- What are the underlying mechanisms/pathways?
- Are there dose-related effects?
- Are there critical windows of vulnerability?
- Effects of poly-substance use?
- Transgenerational effects?
- Effects of paternal substance use?
- Effects of postnatal exposure?



# Linking Prenatal Exposure to Health Outcomes




Prenatal Exposures



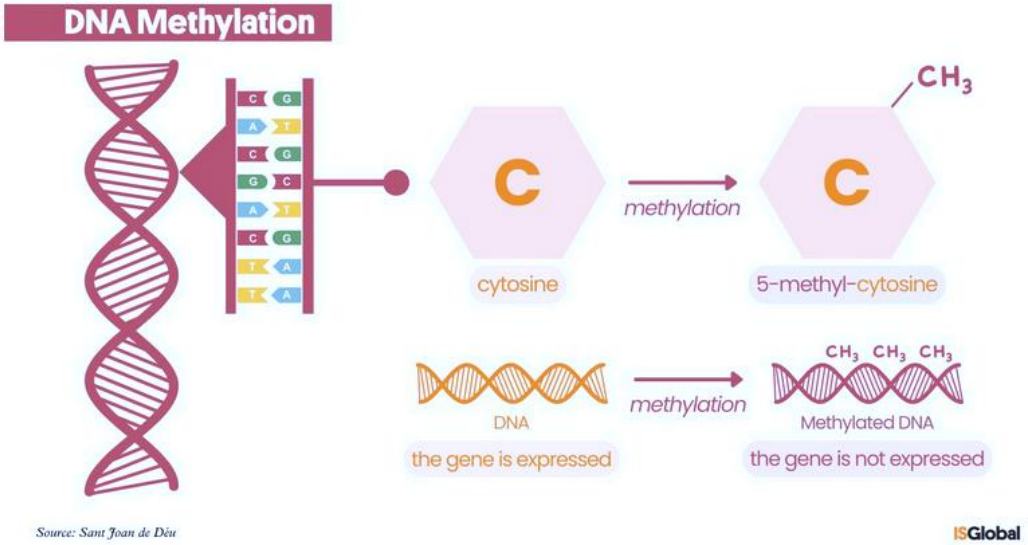

Epigenetics (DNAm)




Health and Disease

# What is DNA methylation (DNAm)?

- Controls how genes are expressed without changing the DNA sequence
- Mainly in CpG context (Cytosine-Guanine)




Eat your dinner!

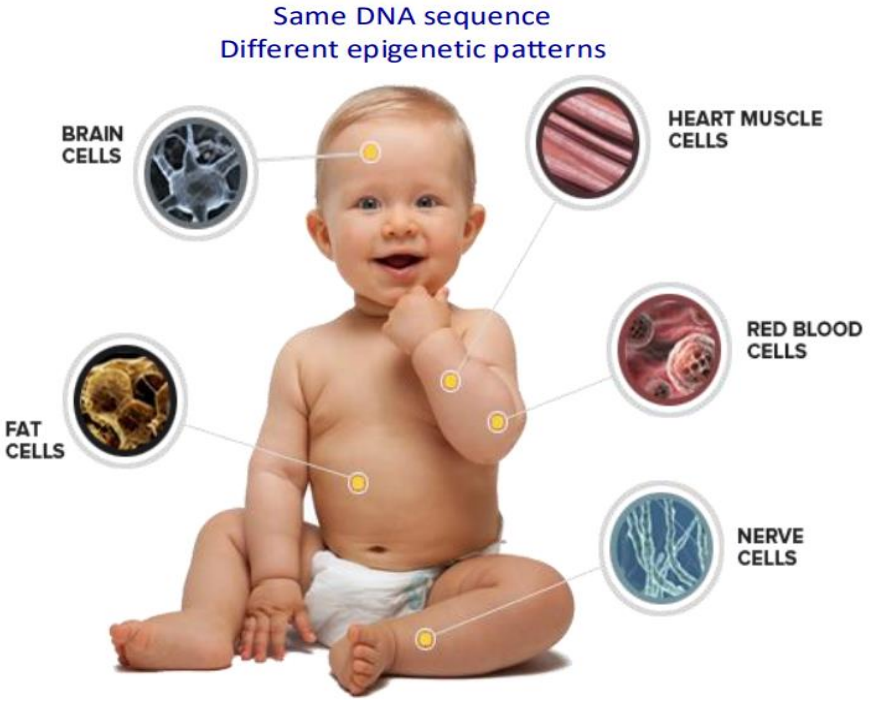


Eat. You're dinner!



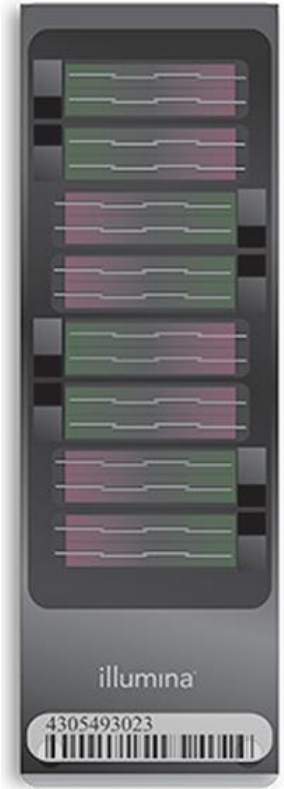
# What is DNA methylation (DNAm)?

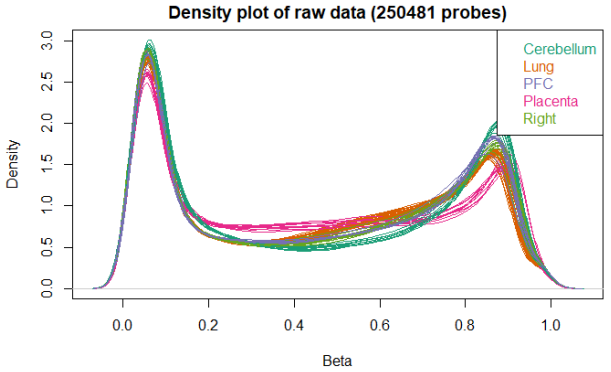
- Essential for normal development
- DNAm is tissue-specific
- Sensitive to nutritional and environmental exposures
- Associated with health and disease
- Maintained across cell divisions and through postnatal development
- Can also be erased, reversible

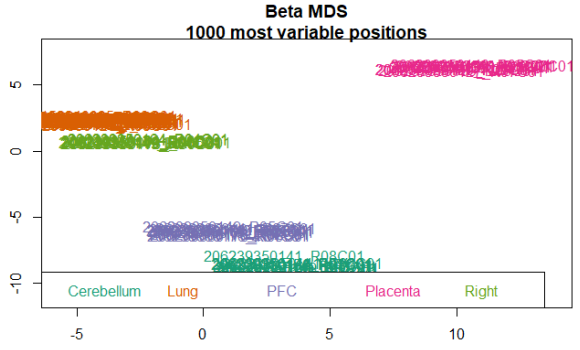


# Illumina DNAm BeadChips

- High-throughput microarray-based platform for DNAm analysis
- Captures information for individual CpG sites across the genome (~27K, 450K, >850K, ~930K CpG sites as technology improves)
- Most used platform in human epidemiological studies

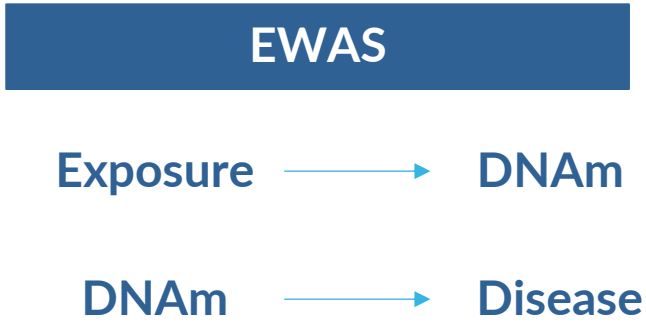


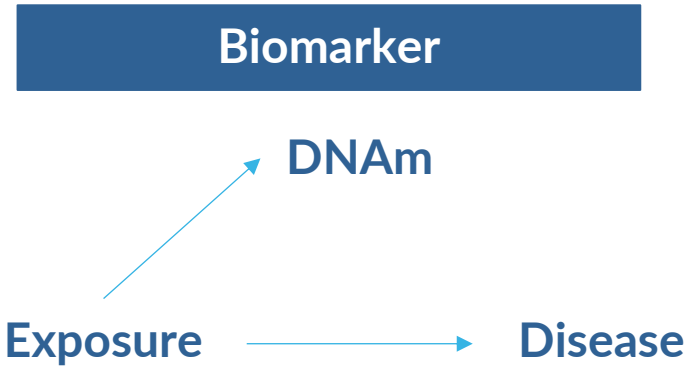



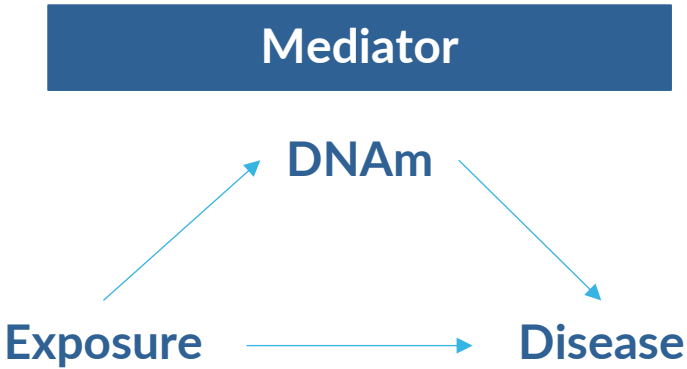



# Utility of DNAm

- Epigenome-wide association studies (EWAS)
  - May help identify biological pathways involved in disease etiology
- Biomarker of exposure and/or disease risk
- Mediation of the exposure-disease relationship



# Utility of DNAm biomarkers

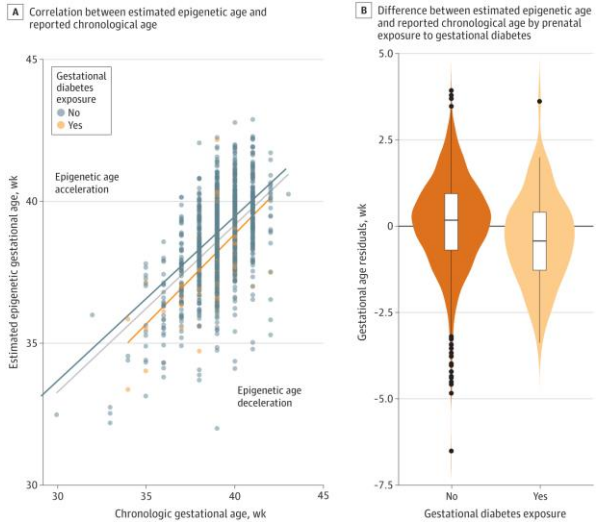
- DNAm can be used to generate "methylation scores"
  - For each sample, we calculate the sum of methylation levels over a selected number of CpG sites, weighted by effect sizes
  - Like polygenetic risk scores which can be used to estimate a person's statistical likelihood of certain diseases or exposures
- Example of "DNAm clock"
- In ECHO, prenatal exposure to maternal gestational diabetes and preeclampsia is associated with delayed biological aging/maturity

JAMA Network Open

Original Investigation | Genetics and Genomics

**Analysis of Pregnancy Complications and Epigenetic Gestational Age of Newborns**

Christine Ladd-Acosta, PhD; Elizabeth Vang, BHSc, BASc; Emily S. Barrett, PhD; Catherine M. Bulka, PhD, MPH; Nicole R. Bush, PhD; Andres Cardenas, PhD, MPH; Dana Dabelea, MD, PhD; Anne L. Dunlop, MD, MPH; Rebecca C. Fry, PhD; Xingyu Gao, MPH; Jaclyn M. Goodrich, PhD; Julie Herbstman, PhD, ScM; Marie-France Hivert, MD, MMSc; Linda G. Kahn, PhD, MPH; Margaret R. Karagas, PhD, MS; Elizabeth M. Kennedy, PhD; Anna K. Knight, PhD; Sahra Mohazzab-Hosseinian, MHS; Andréanne Morin, PhD; Zhongzheng Niu, PhD; T. Michael O'Shea, MD, MPH; Meredith Palmore, MHS; Douglas Ruden, PhD; Rebecca J. Schmidt, PhD; Alicia K. Smith, PhD; Ashley Song, PhD; Eliot R. Spindel, MD, PhD; Leonardo Trasande, MD, MPP; Heather Volk, PhD, MPH; Daniel J. Weisenberger, PhD; Carrie V. Breton, ScD, MPH; for the Environmental Influences on Child Health Outcomes Program



# Prenatal Substance Exposure and DNAm

- Majority of existing studies are for prenatal tobacco exposure/smoking
  - Prior to 2012, studies were mostly targeted (gene specific)
- Limited EWAS of other substances (alcohol, cannabis, opioids)
  - Recent EWAS (2024) of prenatal cannabis not included
  - ECHO analysis of prenatal opioid exposure (limited to 15 CpGs)

| Substance | n studies | n samples (min-max) |
|---|---|---|
| Tobacco | 25 | 14-1,147 |
| Alcohol | 2 | 164-479 |
| FASD | 2 | 6-110 |

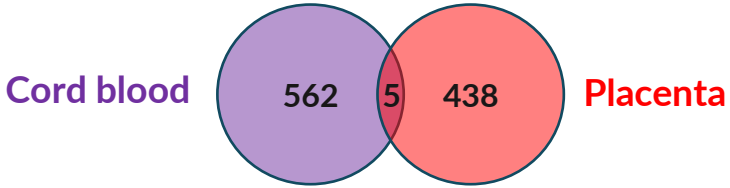
Summarized from Knopik et al. *Neurotoxicol Teratol*. 2019

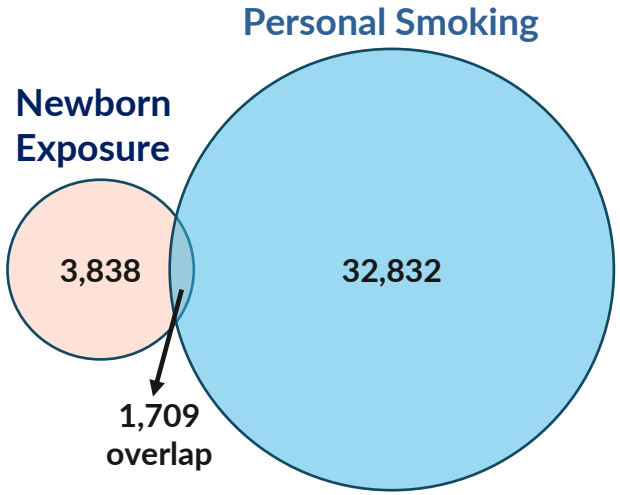
| Substance | n EWAS | n samples (min-max) |
|---|---|---|
| Tobacco | 19 | 20-6,685 |
| Alcohol | 3 | 149-1,018 |
| Opioid | 1 | 96 |

Summarized from Bakulski et al. *Environ Health Perspect*. 2023

# Prenatal Tobacco Exposure and DNAm

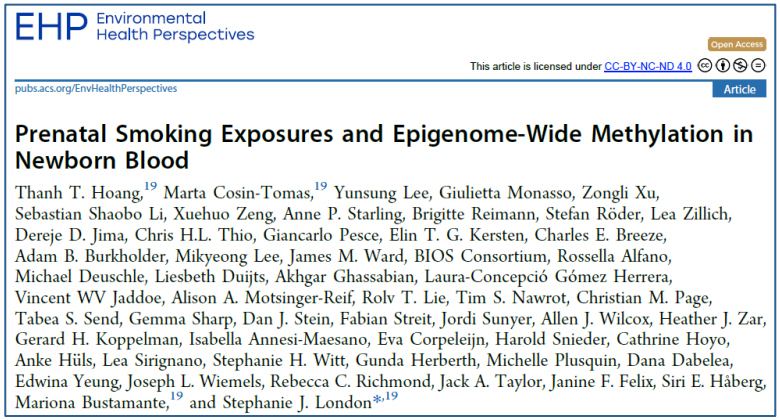
- One of the most extensively studied exposures in environmental epigenetics
- Most EWAS studies in blood DNA (cord blood, child blood) > placenta > other tissues (brain, buccal, lung)
- Results have been replicated across several studies
- Prenatal tobacco exposure DNAm signatures at birth are:
  - Tissue specific
  - Distinct from personal exposure
  - Persistent in later life




Cosin-Tomas, et. al.: Current Environmental Health Reports. 2022
Sikdar et al., Epigenomics. 2019

# Prenatal Tobacco Exposure and DNAm



EHP Environmental Health Perspectives

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pubs.acs.org/EnvHealthPerspectives

Article

**Prenatal Smoking Exposures and Epigenome-Wide Methylation in Newborn Blood**

Thanh T. Hoang,[19] Marta Cosin-Tomas,[19] Yunsung Lee, Giulietta Monasso, Zongli Xu, Sebastian Shaobo Li, Xuehuo Zeng, Anne P. Starling, Brigitte Reimann, Stefan Röder, Lea Zillich, Dereje D. Jima, Chris H.L. Thio, Giancarlo Pesce, Elin T. G. Kersten, Charles E. Breeze, Adam B. Burkholder, Mikyeong Lee, James M. Ward, BIOS Consortium, Rossella Alfano, Michael Deuschle, Liesbeth Duijts, Akhgar Ghassabian, Laura-Concepció Gómez Herrera, Vincent WV Jaddoe, Alison A. Motsinger-Reif, Rolv T. Lie, Tim S. Nawrot, Christian M. Page, Tabea S. Send, Gemma Sharp, Dan J. Stein, Fabian Streit, Jordi Sunyer, Allen J. Wilcox, Heather J. Zar, Gerard H. Koppelman, Isabella Annesi-Maesano, Eva Corpeleijn, Harold Snieder, Cathrine Hoyo, Anke Hüls, Lea Sirignano, Stephanie H. Witt, Gunda Herberth, Michelle Plusquin, Dana Dabelea, Edwina Yeung, Joseph L. Wiemels, Rebecca C. Richmond, Jack A. Taylor, Janine F. Felix, Siri E. Håberg, Mariona Bustamante,[19] and Stephanie J. London*,[19]

PACE

Pregnancy And Childhood Epigenetics

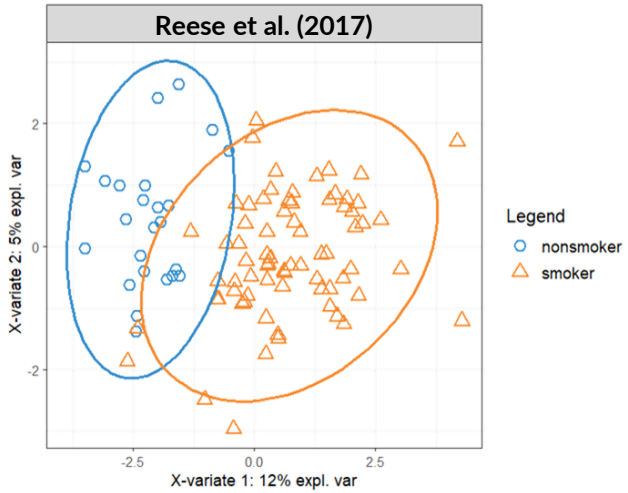
- Meta-analysis of 19 cohorts (n=11,175 parent–newborn pairs)
- Smoking information mostly from questionnaires
- Subsets of cohorts with information on cotinine, former smoking, paternal smoking, second-hand exposure

**Sustained smoking during pregnancy associated with differential DNAm at 8,862 CpGs**

- Did not differ by infant sex
- Enriched for regions that regulate transcription in blood, placenta, lung, and heart

# Prenatal Tobacco Exposure DNAm biomarkers

| Study | DNAm age | Tissue | n CpGs | Predictive Accuracy |
|---|---|---|---|---|
| Ladd-Acosta et al. (2016) | Early Child (5 years) | Peripheral blood | 26 | AUC = 0.87 |
| Reese et al. (2017) | Birth | Cord blood | 28 | AUC = 0.90 |
| Richmond et al. (2018) | Adult (~30 years) | Peripheral blood | 568 | AUC = 0.69 |
| Rauschert et al. (2020) | Adolescents (17 years) | Peripheral blood | 204 | AUC = 0.87 |

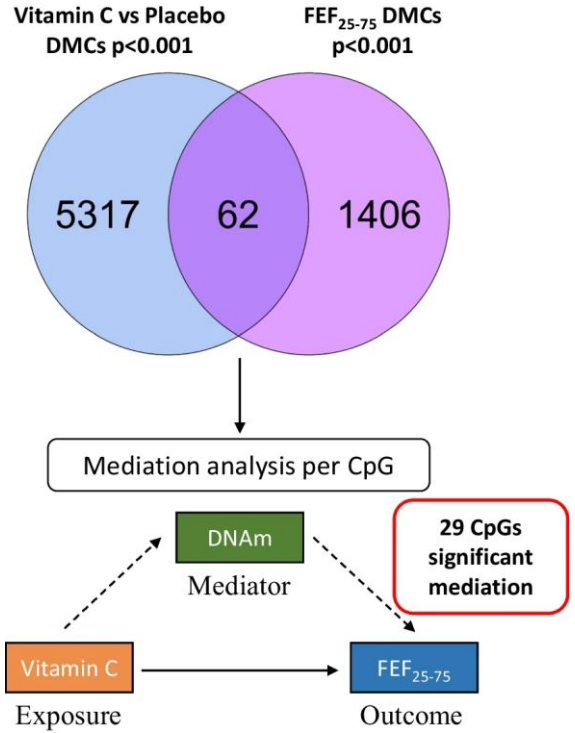



DNA methylation signatures associated with prenatal tobacco exposure have been repeatedly validated and are unique to specific tissues and timing of exposure

# DNAm as a Mediator of Health Outcomes

DNAm partially mediates -

- The effect of **maternal smoking** on offspring **birthweight** in cord blood and placenta
- The effect of **maternal smoking** on **neurodevelopment** at 6 months of age
- The effect of **vitamin C** supplementation on **lung function** at age 5





Xu et al., *Clin Epigenetics.* 2021
Cardenas et al., *Am J Epidemiol.* 2019
Abrishamcar et al., *Transl Psychiatry.* 2022
Shorey-Kendrick et al., *Clin Epi*. 2025

# Prenatal Cannabis and DNAm

www.nature.com/mp

Molecular Psychiatry

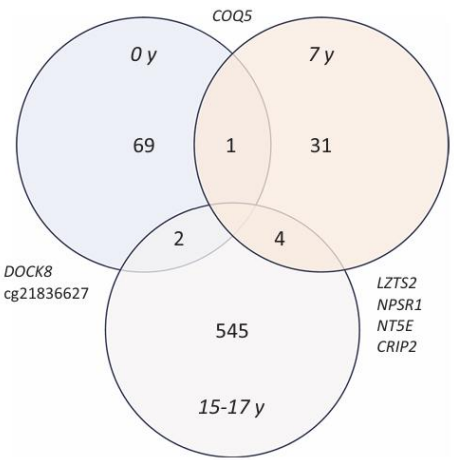
ARTICLE OPEN

Check for updates

## Prenatal cannabis exposure is associated with alterations in offspring DNA methylation at genes involved in neurodevelopment, across the life course

Alexandra J. Noble[1]✉, Alex T. Adams[1,2], Jack Satsangi[1,2], Joseph M. Boden[3] and Amy J. Osborne[4]✉

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Avon Longitudinal Study of Parents and Children (ALSPAC)

**Table 1.** Cohort characteristics for ALSPAC and CHDS.

| Cohort | Age (y) | N | Sample | Female (%) | PCE | PCTE | PTE | Tobacco smoking | Cannabis user |
|---|---|---|---|---|---|---|---|---|---|
| ***ALSPAC*** | | | | | | | | | |
| Timepoint 1 | 0 | 858 | Cord blood | 448 (52.21%) | 10 (1.51%) | 30 (3.14%) | 168 (19.58%) | | |
| Timepoint 2 | 7 | 924 | Whole blood | 473 (51.19%) | 9 (1.08%) | 22 (3.46%) | 184 (19.91%) | | |
| Timepoint 3 | 15-17 | 922 | Whole blood | 484 (52.49%) | 11 (1.19%) | 21 (3.57%) | 182 (19.73%) | | |
| ***CHDS*** | | | | | | | | | |
| Timepoint 1 | ~27 | 98 | Whole blood | 27 (27.5%) | 4 (4.08%) | 13 (13.2%) | 55 (56.1%) | 28 (28.5%) | 37 (37.5%) |

*CHDS* participants (27 y) were also classified for personal tobacco or cannabis usage as adults.
*PCE* prenatal cannabis exposure controlled for prenatal tobacco exposure, *PCTE* prenatal cannabis exposure with tobacco co-exposure, *PTE* prenatal tobacco exposure only.

# Estimated Prenatal Substance Use in ECHO

| Substance | n (%) |
|---|---|
| Alcohol | 7,328 (13.9) |
| Tobacco | 6,969 (10.4) |
| Opioid/pain missuse | 4,899 (11) |
| Cigarette | 4,737 (8.5) |
| Cannabis | 2,148 (6.8) |
| Vape | 1,033 (2.9) |
| Illicit | 421 (1.7) |

- Estimates are based on data harmonization tables in PlatIPUS
- Any/ever use
- % calculated as Yes/(No + Yes)
- Does not include subjects with missing data

(*March 1, 2026 snapshot*)

# Availability of Child DNAm data in ECHO

## WAVE 1: CURRENTLY AVAILABLE

**8,027 samples:**

- 10 different biospecimen types (sample n=142 to 2435 per biospecimen type)
- 4 different versions of the Illumina array (27K, 450K, EPICv1, Asthma&Allergy)

## WAVE 2: CURRENTLY AVAILABLE

**2,132 longitudinal samples** (55 saliva) from **884** participants:

- 2 different biospecimen types: blood, saliva
- Processed on Illumina EPICv2 microarray

## WAVE 3: ESTIMATED MID-JUNE COMPLETION

**11,038 samples** submitted for measurement:

- 2 different biospecimen types: blood, saliva
- Processed on Illumina EPICv2 microarray

Combined (>20,000 child samples)

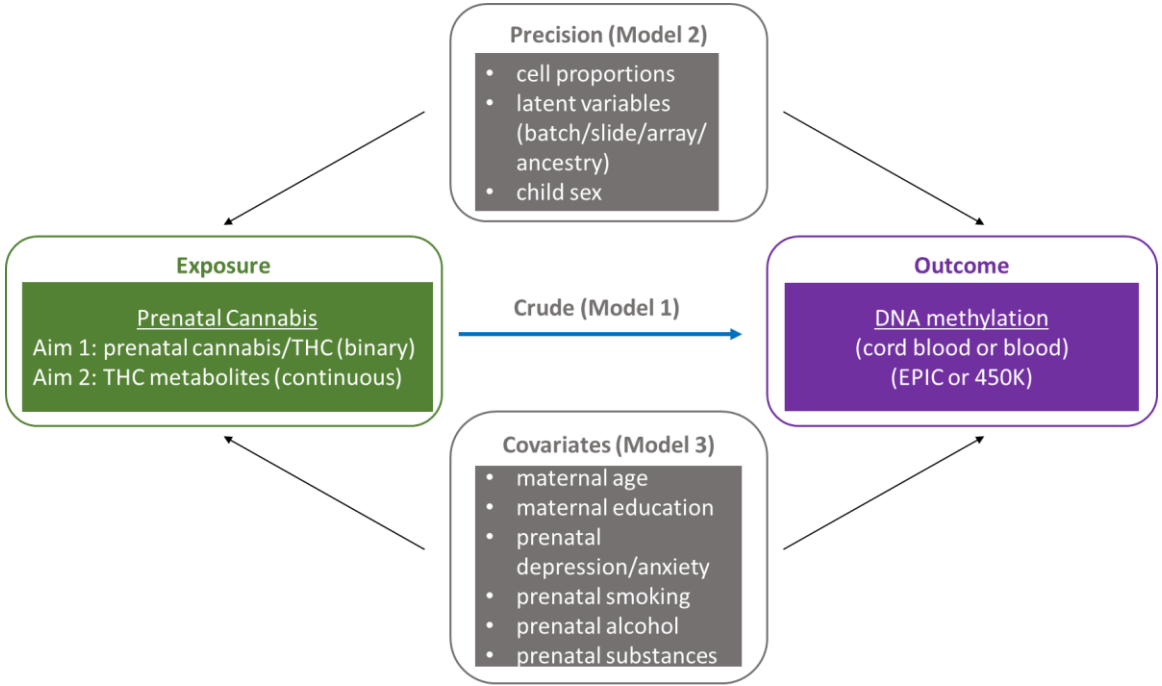
## WAVE 3

| DNA Source | n (%) |
|---|---|
| Blood | 1,658 (14.7%) |
| Buffy Coat | 235 (2.1%) |
| Cord Blood | 918 (8.1%) |
| Saliva | 7,339 (64.8%) |
| Unknown | <5 (<1%) |
| White Blood Cell | <170 (<2%) |
| Whole Blood | 738 (6.5%) |

# Prenatal Cannabis and DNAm in ECHO

EC0809

- DNAm measures from cord blood or newborn blood spots
- Maternal cannabis use data during the index pregnancy
- 900 mother-child pairs from 8 cohort sites
- Prevalence of prenatal cannabis = 3.7% (n=34 exposed)



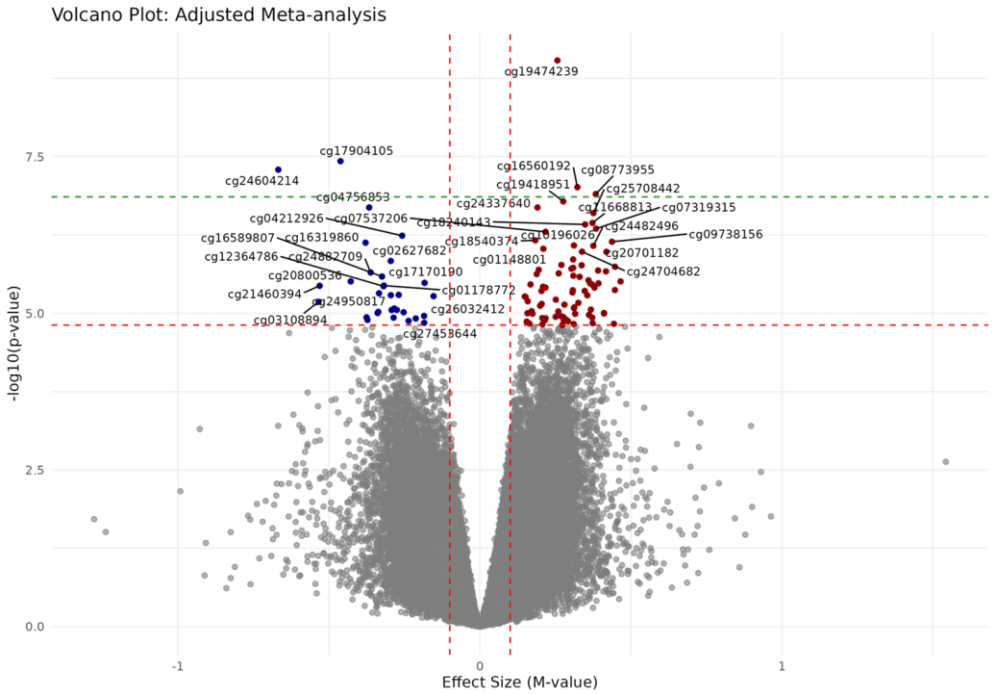
# Prenatal Cannabis and DNAm in ECHO

EC0809

| | Primary Model* |
|---|---|
| DMCs (FDR < 0.05) | 112 (99 genes) |
| Number of DMRs | 23 (25 genes) |

## Enriched for genes involved in:

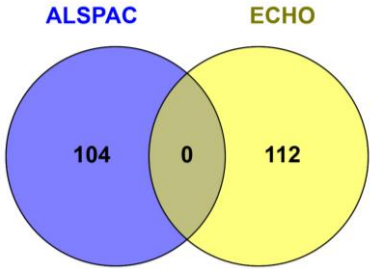
- Schaffer collateral - CA1 synapse
- Regulation of eating behavior
- Epigenetic regulation of gene expression




* adjusted for infant sex, maternal age, maternal education, use, alcohol use, federally illicit drug use (other than cannabis), and surrogate variables capturing cell composition and ancestry

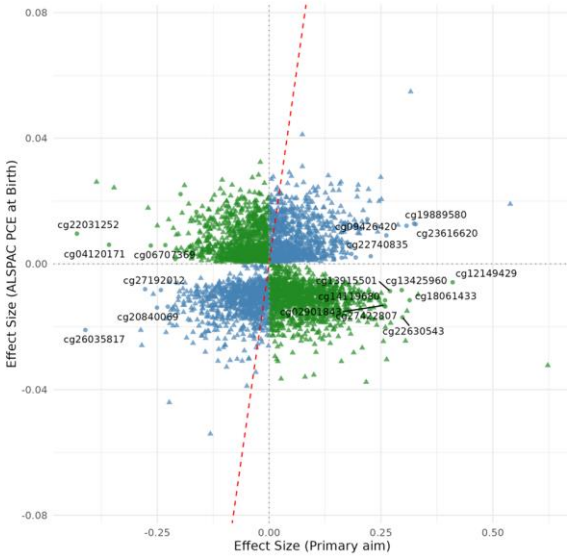
# Comparison between ALSPAC and ECHO EC0809

Both studies found enrichment for differential methylation in biological pathways related to neurodevelopment, neuronal structure and function




No overlap of FDR CpGs

Increased methylation of *PCYOX1* in both studies




Lookup of top 5,000 ALSPAC CpGs

52.9% of available CpGs associated with prenatal cannabis exposure in the same direction

## ALSPAC (Avon Longitudinal Study of Parents and Children)

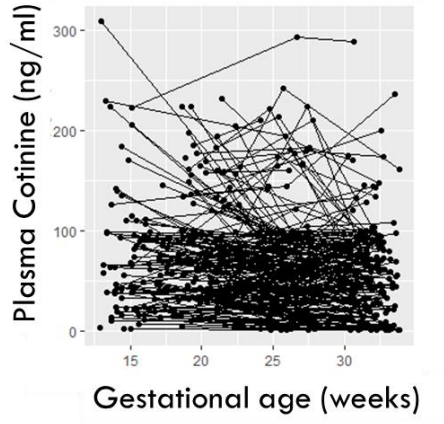
- Avon, United Kingdom
- Birth years 1991 – 1992
- Higher SES
- 97.8% white
- Cannabis illegal

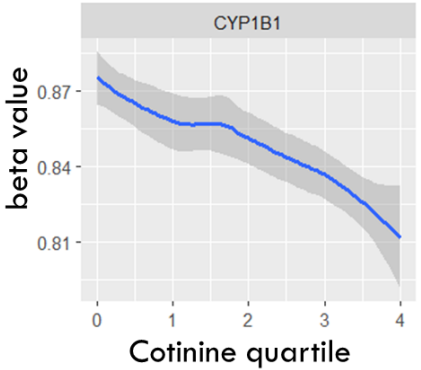
## ECHO (this study)

- USA multiple states
- Birth years 1995 – 2020
- 58% white
- Changing legal landscape
- Increasing THC concentration

# Development of DNAm biomarkers in ECHO

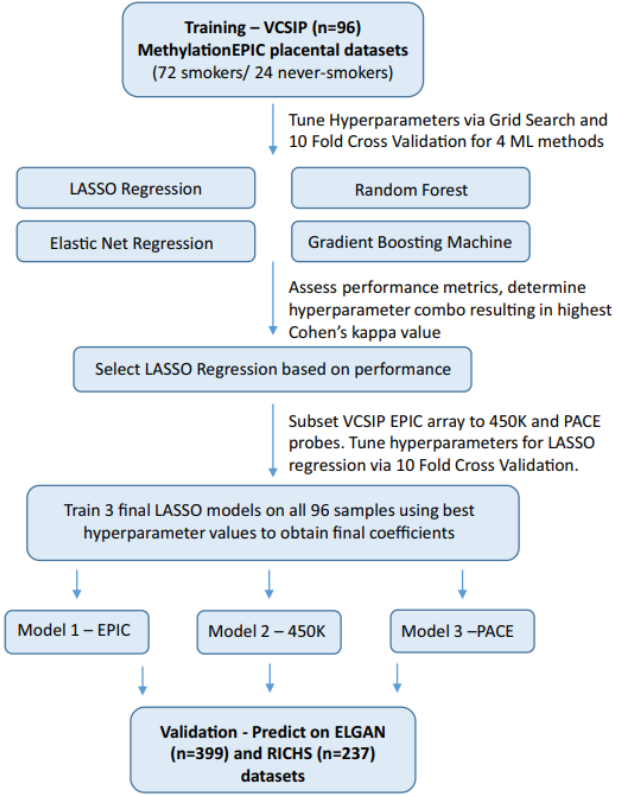
- Cotinine is the primary metabolite of nicotine and the gold standard chemical biomarker of daily smoking
- Single or cross-sectional cotinine measures are unreliable measures of cumulative prenatal smoke exposure, because daily smoking can fluctuate greatly
- DNAm associated with maternal smoke is dose-dependent at some CpGs



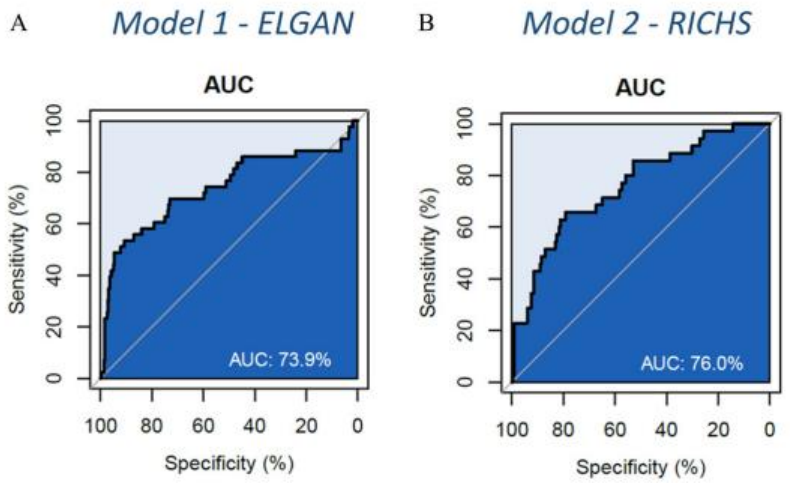

Shorey-Kendrick et al.,*Clin Epigenetics*. 2021

# Development of DNAm biomarkers in ECHO




**Development and Validation of a Novel Placental DNA Methylation Biomarker of Maternal Smoking during Pregnancy in the ECHO Program**

*Lyndsey E. Shorey-Kendrick,[1] Brett Davis,[2] Lina Gao,[3,4] Byung Park,[3,4,5] Annette Vu,[6] Cynthia D. Morris,[6,7] Carrie V. Breton,[8] Rebecca Fry,[9] Erika Garcia,[8] Rebecca J. Schmidt,[10,11] T. Michael O'Shea,[12] Robert S. Tepper,[13] Cindy T. McEvoy,[14] and Eliot R. Spindel,[1] on behalf of program collaborators for Environmental influences on Child Health Outcomes**

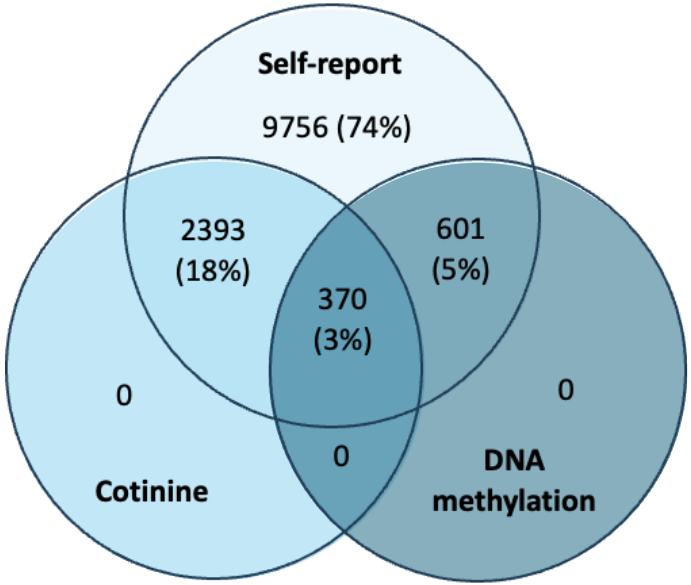



Shorey-Kendrick et al.,*EHP*. 2024

# Utility of DNAm biomarkers in ECHO

EC0533

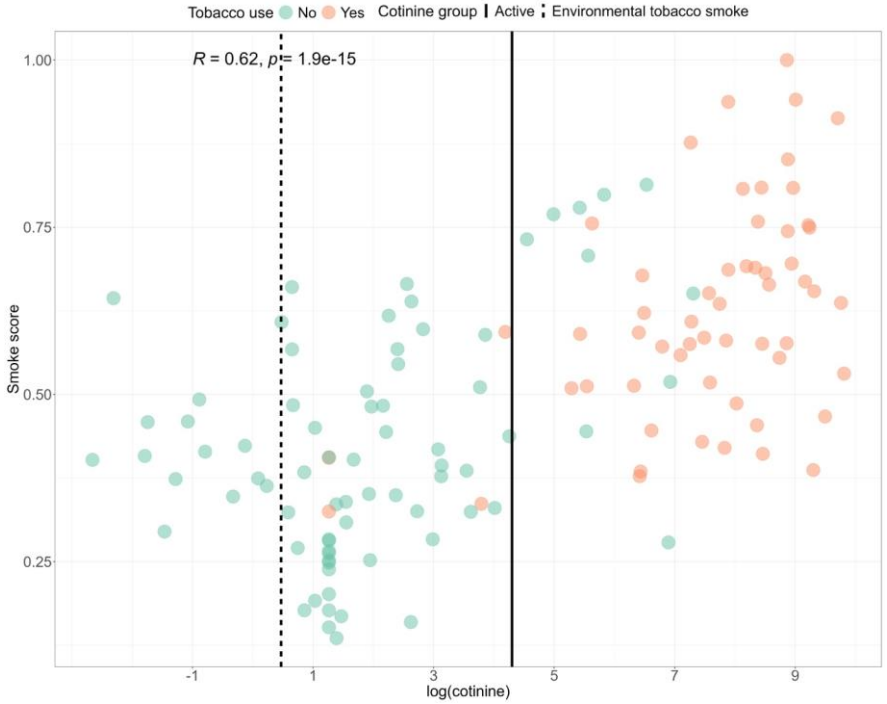
- Previous studies suggest that prenatal exposure to tobacco smoking is associated with increased offspring blood pressure
  - Findings have been inconsistent and mainly limited to self-report of smoking during pregnancy
- We evaluated this association in ECHO using:
  - Any report of prenatal tobacco smoking
  - Pregnancy urinary cotinine
  - DNAm based biomarker in child blood




Shorey-Kendrick et al.,*Circulation*. 2026

# Utility of DNAm biomarkers in ECHO

EC0533




| | Systolic blood pressure (Z score) | | Diastolic blood pressure (Z score) | |
|---|---|---|---|---|
| | **Effect estimate (95% CI)** | ***P* value** | **Effect estimate (95% CI)** | ***P* value** |
| **Any prenatal tobacco exposure** (n=13120 SBP; 13083 DBP) | | | | |
| *Categorical self-report: yes/no* | | | | |
| | 0.021 (-0.025, 0.069) | 0.388 | 0.196 (0.155, 0.237) | **<0.001** |
| **Pregnancy urine cotinine** (n=2763 SBP; 2726 DBP) | | | | |
| *Categorical cotinine: Active vs no exposure* | | | | |
| | 0.215 (0.122, 0.308) | **<0.001** | 0.417 (0.341, 0.492) | **<0.001** |
| **DNAm PrenatalSmokeScore** (n=971) | | | | |
| *Categorical methylation: active versus no exposure* | | | | |
| | 0.142 (0.029, 0.255) | **0.014** | 0.065 (-0.027, 0.157) | 0.167 |

[1]Adjusted for child race, maternal age at delivery, maternal pre-pregnancy BMI, prenatal

[2] Passive exposure was defined as having a cotinine level between 1.6 and 74 ng/ml, and active exposure for cotinine measures greater than or equal to 75 ng/ml

Shorey-Kendrick et al.,*Circulation*. 2026

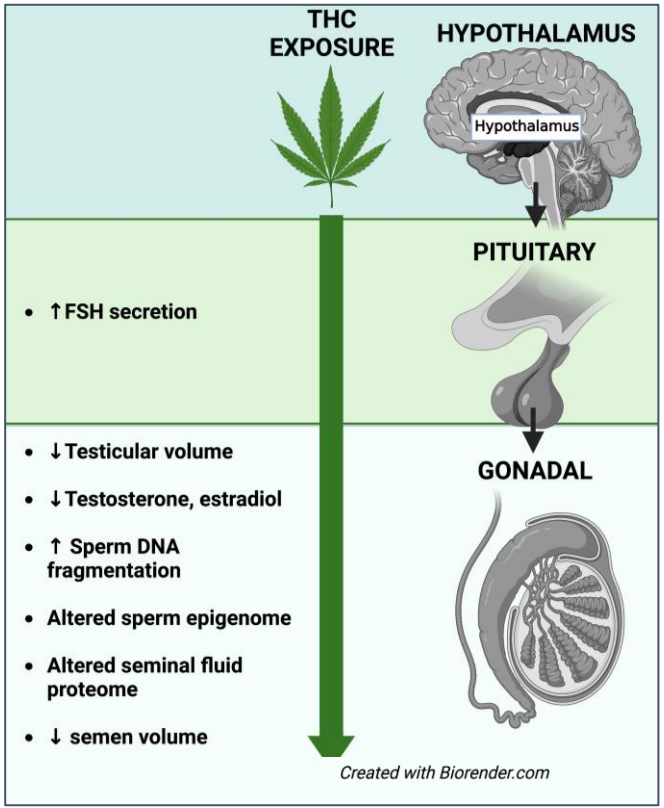
# Data Available in ECHO

- >20,000 DNAm datasets from multiple tissues
- Newly available paired genetic and DNAm data
- Metabolomic and bioassay data
- Cycle 2
  - Collection of exposure data during pregnancy avoids recall bias
  - Information on child's own substance use
- Siblings with discordant prenatal substance exposure
- Incoming paternal biospecimens (semen/sperm)



# Effects of Paternal Substance Use

- **Paternal environmental** exposures, lifestyle, and health status preconception can influence offspring health and disease risk
- Advanced paternal age (>40 yo) is associated with increased risk of preterm birth, small for gestational age
- Sperm DNA integrity is associated with small for gestational age / low birth weight
- Evidence from human and animal studies that paternal substance use impacts reproductive and child health outcomes





Li et al. *Sci Rep* 2024
Khandwala et al. *BMJ* 2018
Boeri et al. *World J MensHealth* 2024
Lo et al., *Nature Reviews Urology* 2024

# Estimated Child Substance Use in ECHO

| Substance | n (%) |
|---|---|
| Alcohol | 6,061 (36.3) |
| Cannabis | 3,415 (22.7) |
| Vape | 3,344 (19.9) |
| Tobacco | 3,904 (9.5) |
| Cigarette | 1,454 (8) |
| Opioid/pain missuse | 623 (5.5) |
| Illicit | 305 (2.7) |

- Estimates are based on data harmonization tables in PlatIPUS
- Any/ever use
- % calculated as Yes/(No + Yes)
- Does not include subjects with missing data

At what age did you first consume alcohol?

On average, in the past 12 months, how often did you consume alchohol?

*(March 1, 2026 snapshot)*

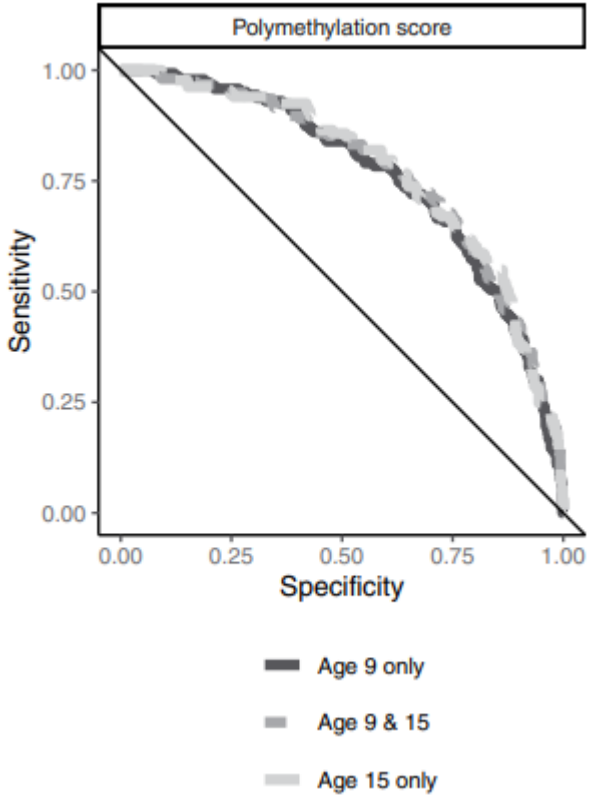
# Cross-tissue Utility of Polymethylation Scores

EPIGENETICS
2022, VOL. 17, NO. 13, 2223–2240
https://doi.org/10.1080/15592294.2022.2112815

**Polymethylation scores for prenatal maternal smoke exposure persist until age 15 and are detected in saliva in the Fragile Families and Child Wellbeing cohort**

Freida A. Blostein[a], Jonah Fisher[b], John Dou[a], Lisa Schneper[c], Erin B. Ware[c], Daniel A. Notterman[c], Colter Mitchell[b*], and Kelly M. Bakulski [a*]

- Results from a meta-analysis of prenatal smoke exposure and DNAm in newborn cord blood were used to create polymethylation scores (PMS) of child saliva DNA
- Prenatal smoke exposure was significantly associated with a higher PMS in European and African genetic ancestry samples
- The PMS classified prenatal smoke exposure accurately (AUC age 9 = 0.77, age 15 = 0.76)
- PMS from saliva samples could serve as robust and practical biomarkers of prenatal smoke exposure



# Summary and Implications for ECHO

- **Additional EWAS**
  - Identify biological pathways associated with exposure or outcomes
- **Use of existing DNAm biomarkers**
  - Proxy for prenatal tobacco exposure for association with outcomes
  - Examine DNAm as a mediator between exposure and outcomes
  - Explore cross-tissue utility (e.g. blood-based biomarkers in saliva DNAm)
- **Development of new DNAm biomarkers**
  - Prenatal substance exposure
    - Incorporate bioassay or metabolomic data
  - Child's own substance use
    - Utilize longitudinally collected samples and new onset of substance use
- **Effects of paternal substance use on DNAm (sperm and child)**



# Thank You!