What is DNA methylation and how can it help us address ECHO scientific priorities and improve child health?

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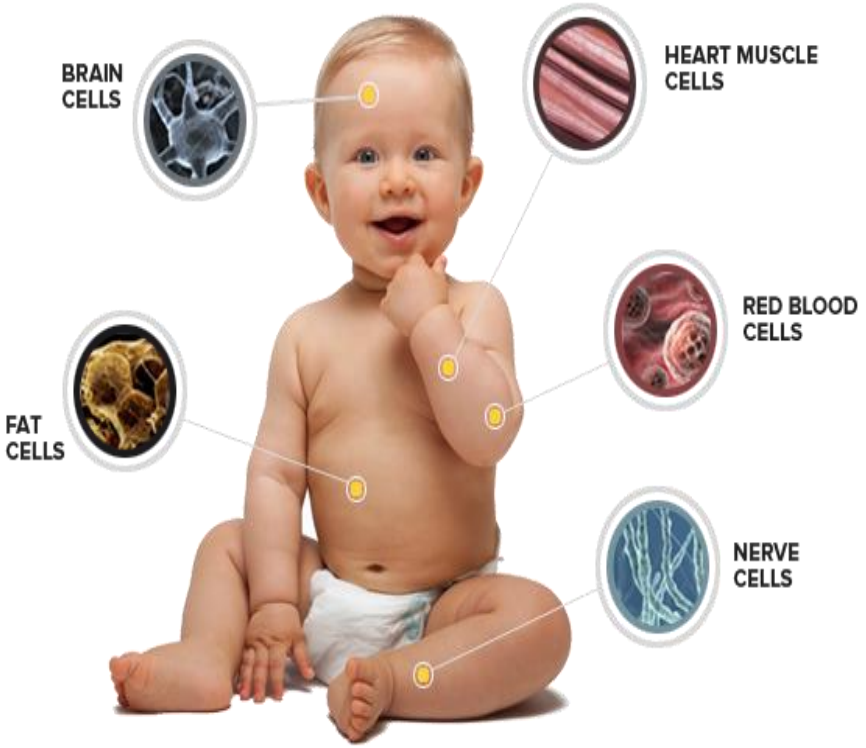
ECHO-DAC

What is DNA methylation and why is it important?

What is DNA methylation?

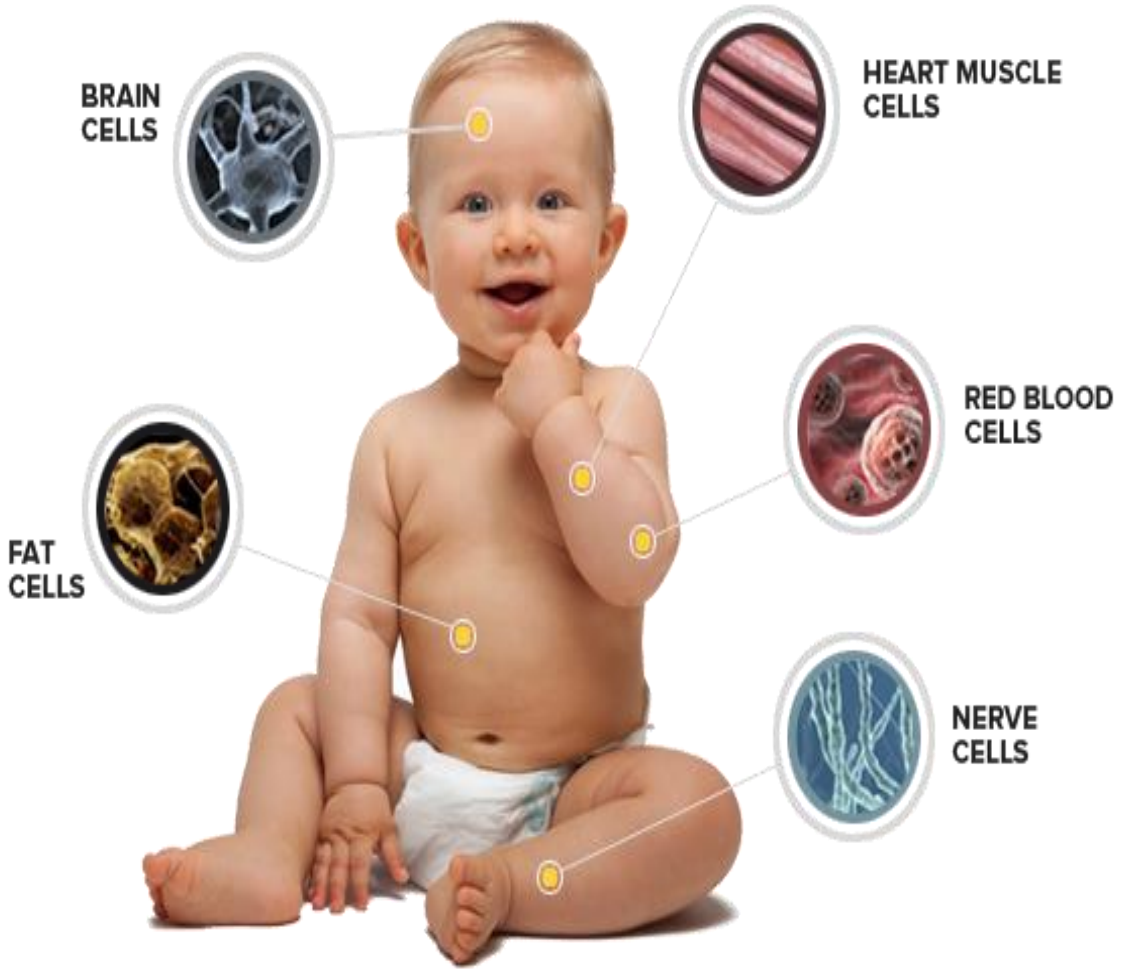
DNA methylation is a kind of biologic material that provides a mechanism for cells with the same genotype to have different identities and functions



SAME DNA sequence/genes
DIFFERENT DNA methylation patterns

DNA methylation is mitotically heritable

Same DNA sequence
Different epigenetic patterns

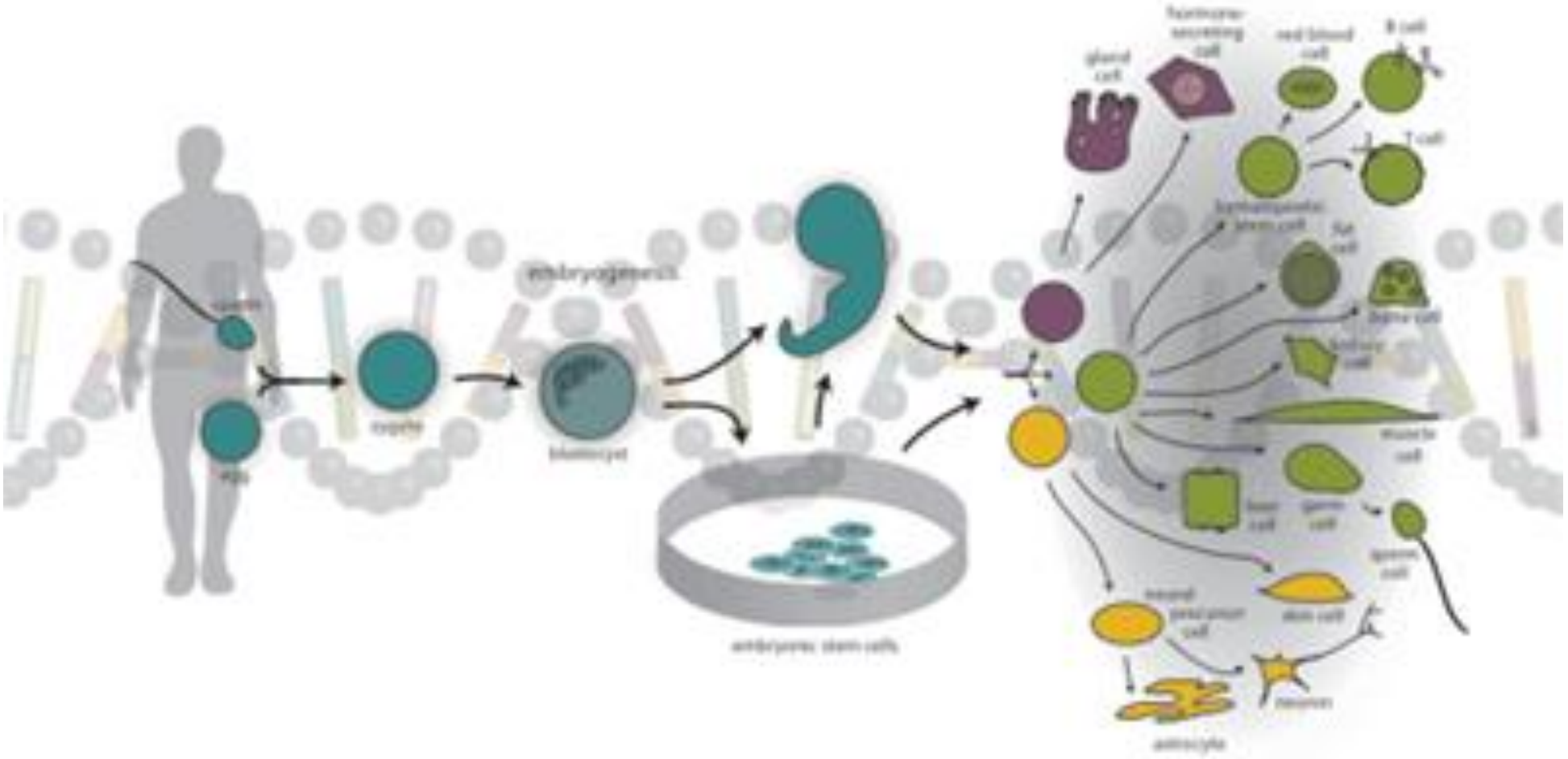


DNA methylation provides a mechanism for cells to remember their cell lineage when they divide, i.e. undergo mitosis

mitosis

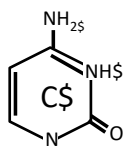
cell type remembered

DNA methylation plays a key role in organismal development

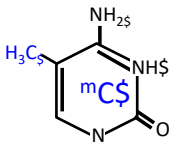


Large, dynamic changes occur during the developmental window

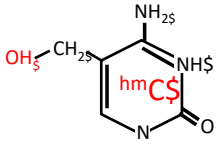
The term “DNA methylation” is used to describe the covalent addition of a methyl or hydroxymethyl group to cytosine nucleotides (C) in the DNA sequence



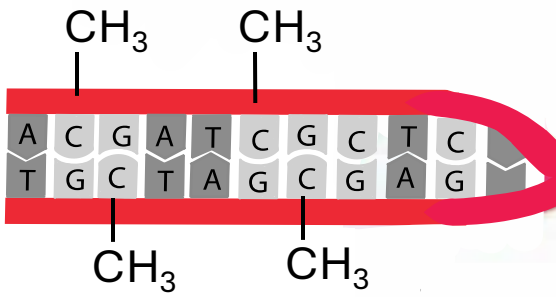
cytosine



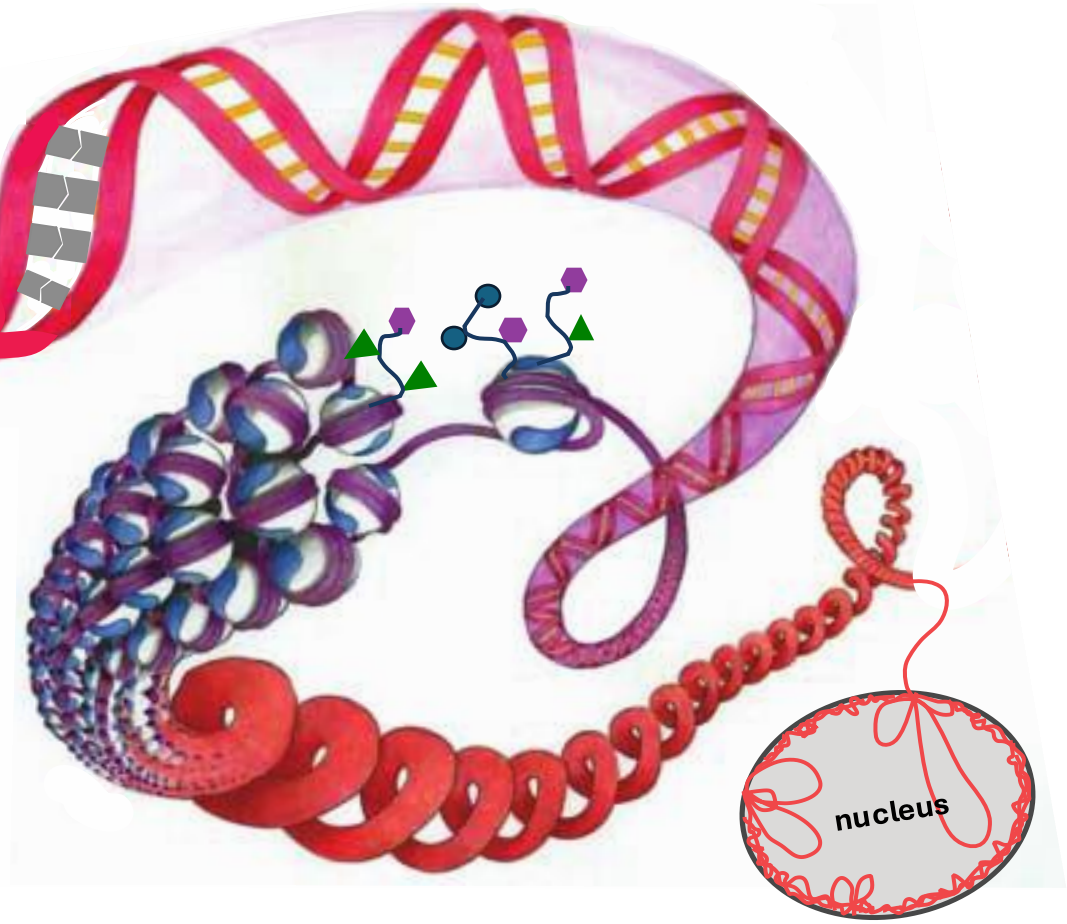
5-methylcytosine



5-hydroxymethylcytosine



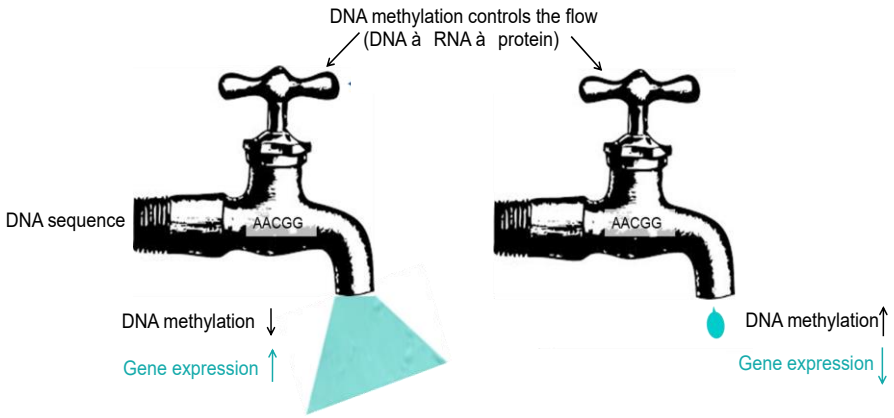
DNA methylation (DNAm)



- Mainly occurs at CpG dinucleotides in humans
- About 28 million CpGs
- Hydroxymethylation mostly in brain and stem cells

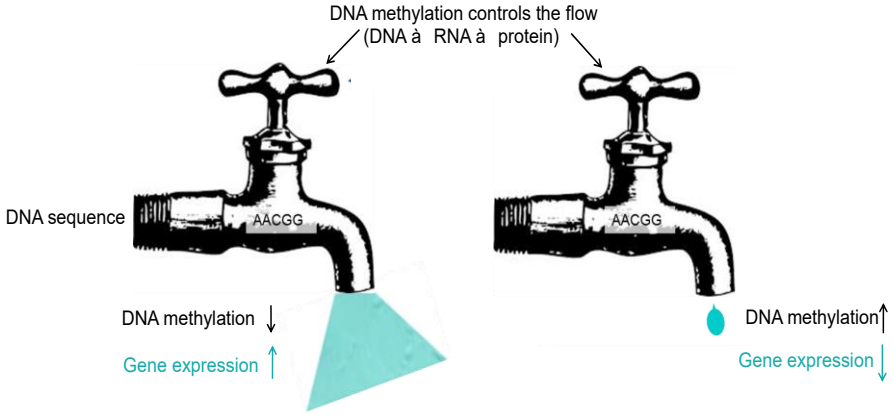
DNA methylation is critical to multiple cell functions

1. Gene expression regulation

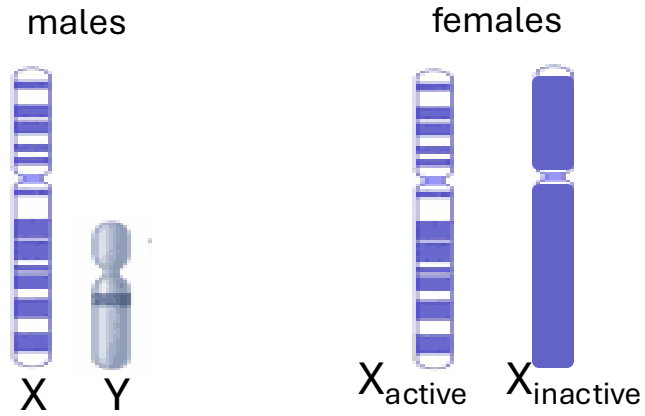


DNA methylation is critical to multiple cell functions

1. Gene expression regulation



2. X chromosome dosage compensation



DNA methylation is critical to multiple cell functions

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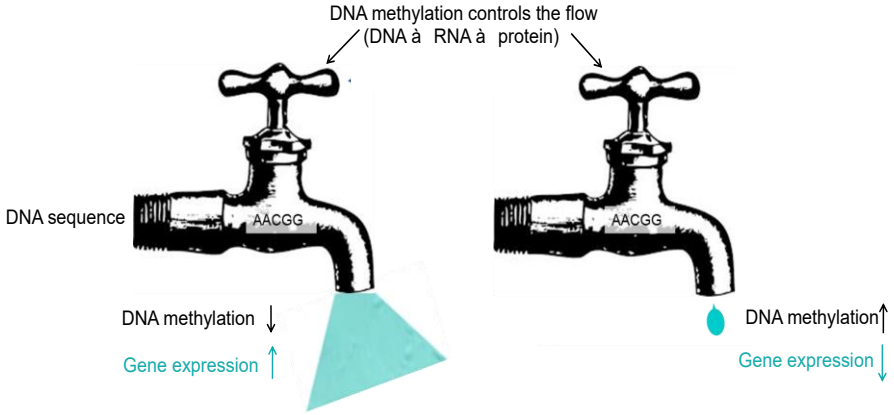

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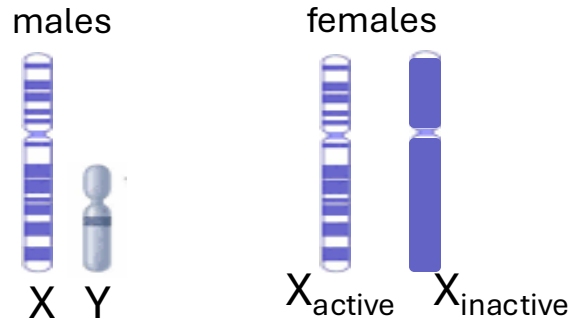
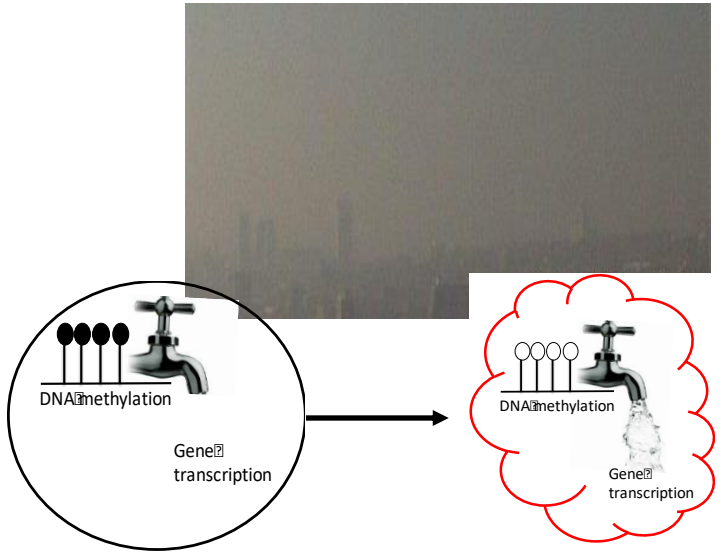


3. Response to environment without change in DNA sequence



DNA methylation is critical to multiple cell functions

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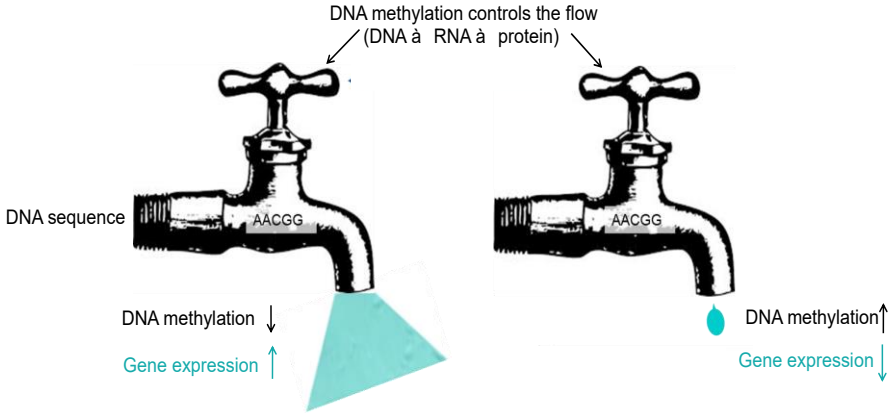

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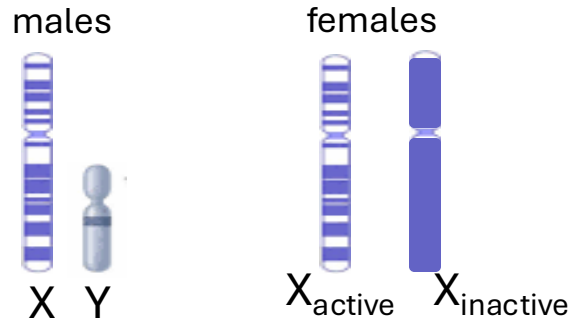

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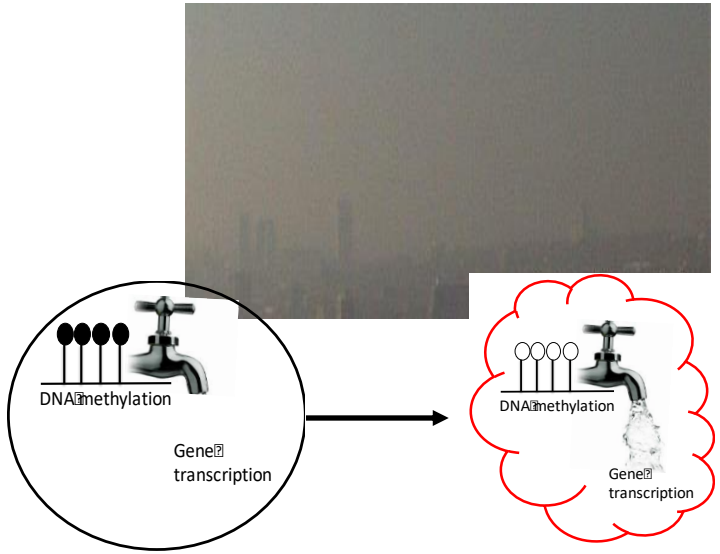
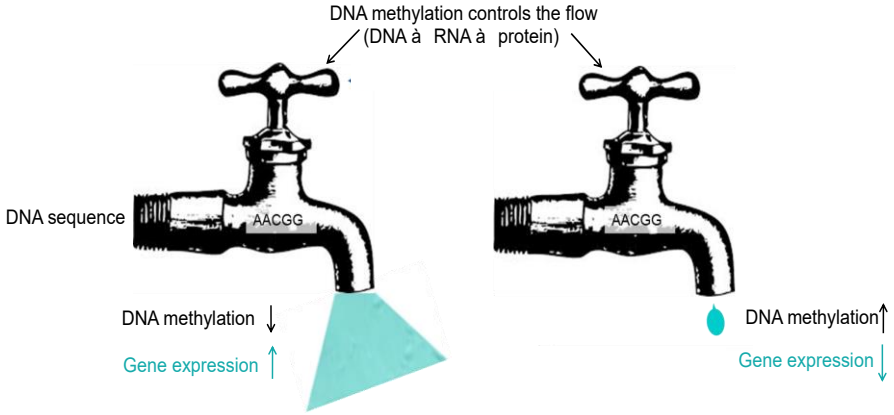


4. Chromosomal stability

Silence repetitive DNA elements

DNA methylation is critical to multiple cell functions

1. Gene expression regulation



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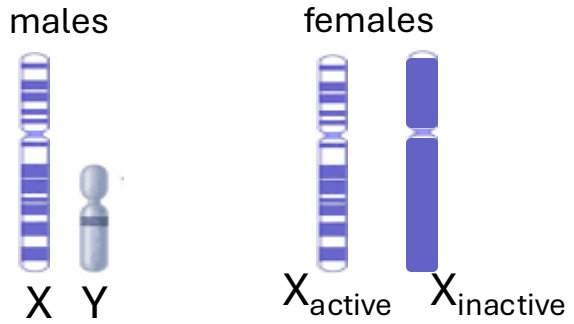
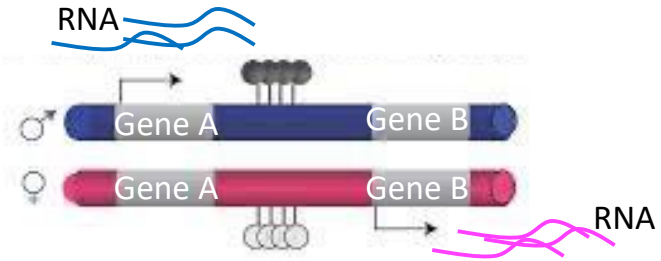


5. Imprinting

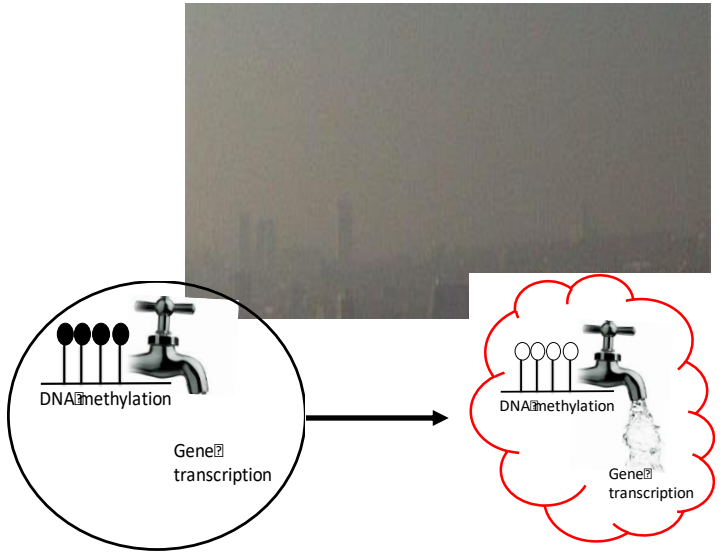


Epigenetic patterns mark which copy of the DNA was inherited from each parent
→ results in differential allele expression

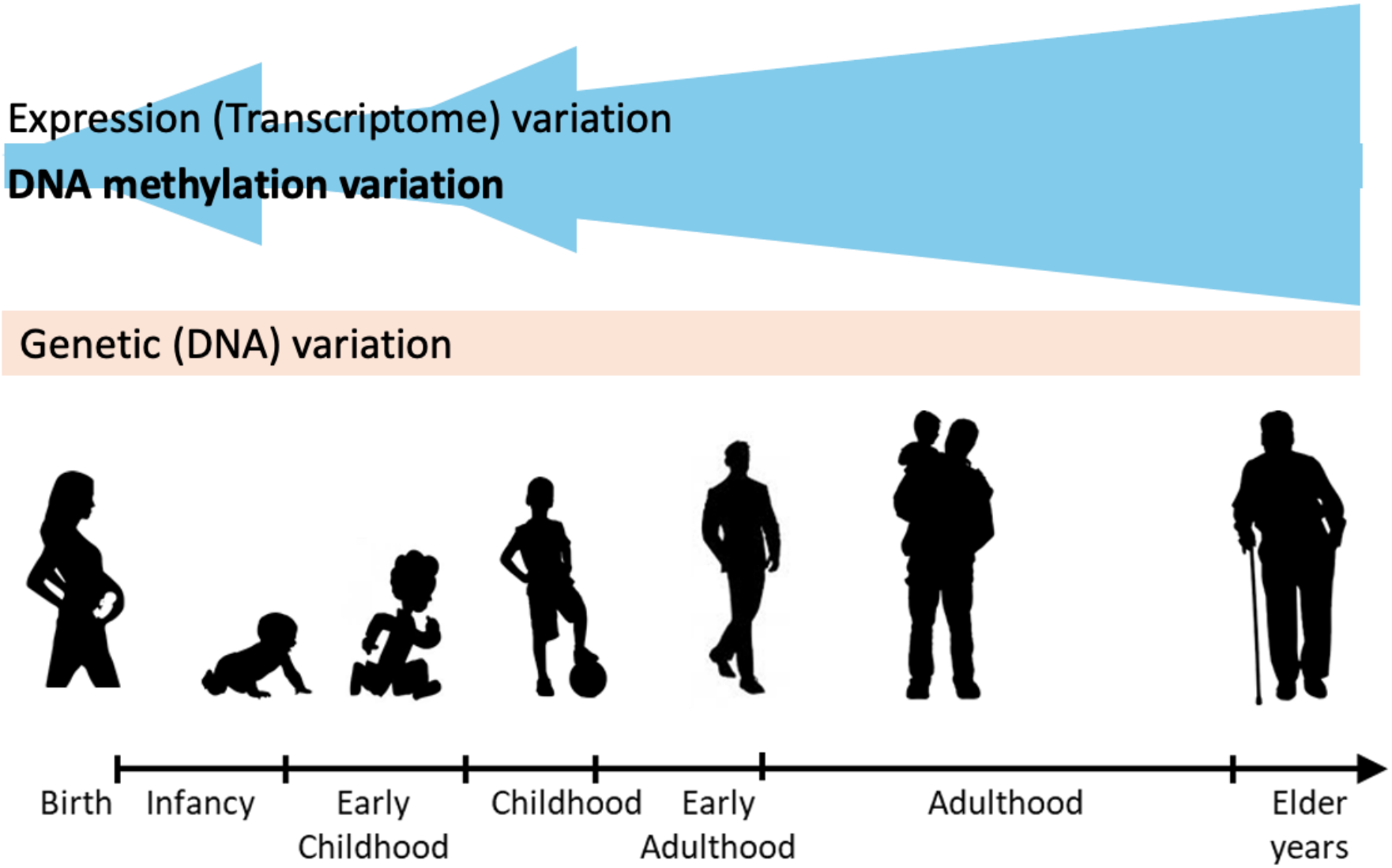
4. Chromosomal stability

Silence repetitive DNA elements

3. Response to environment without change in DNA sequence



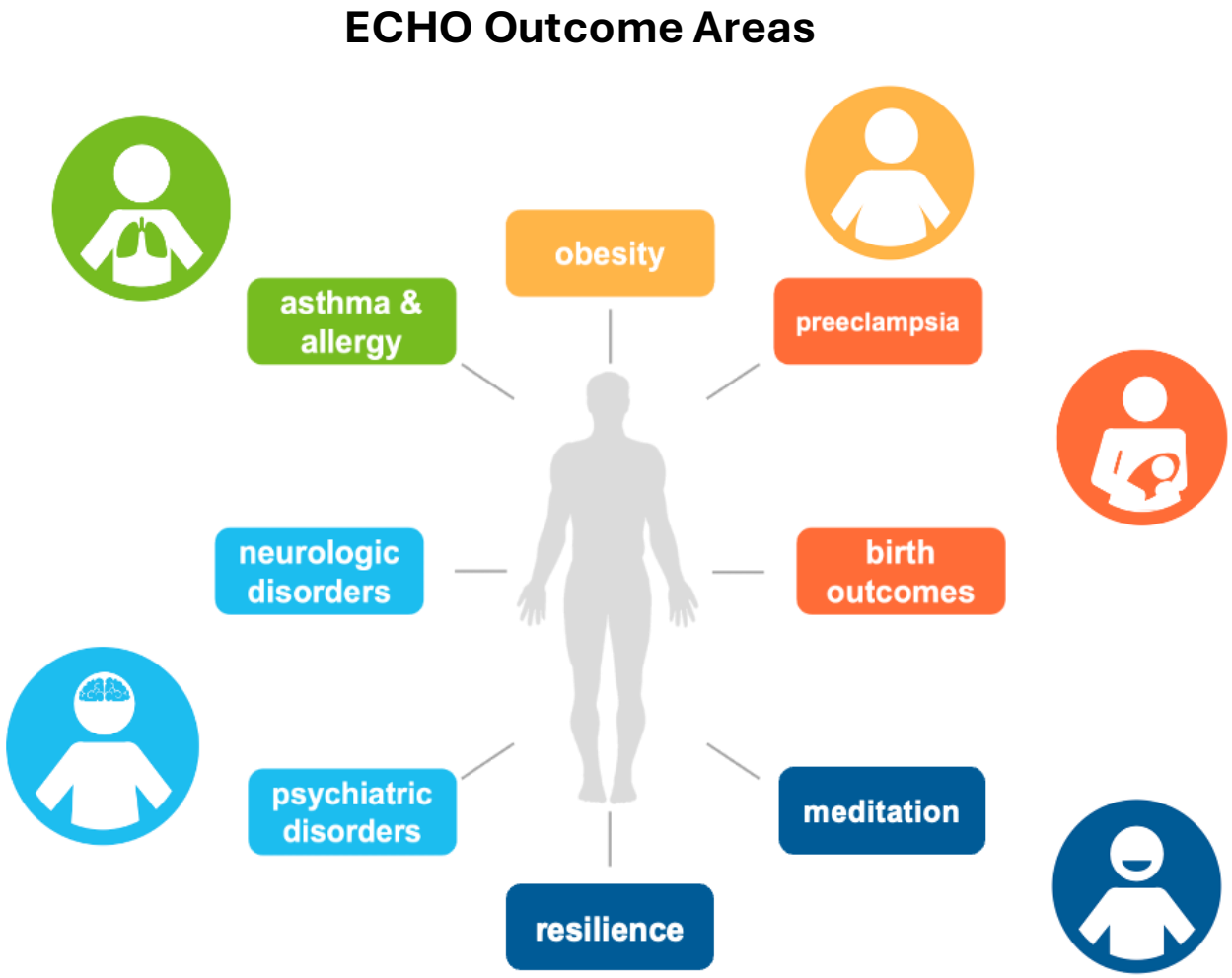
While genes are static,
(some) DNA methylation patterns can change over time



DNA methylation changes have been associated with a range of human health outcomes

Empirical evidence, changes in DNA methylation associated with:

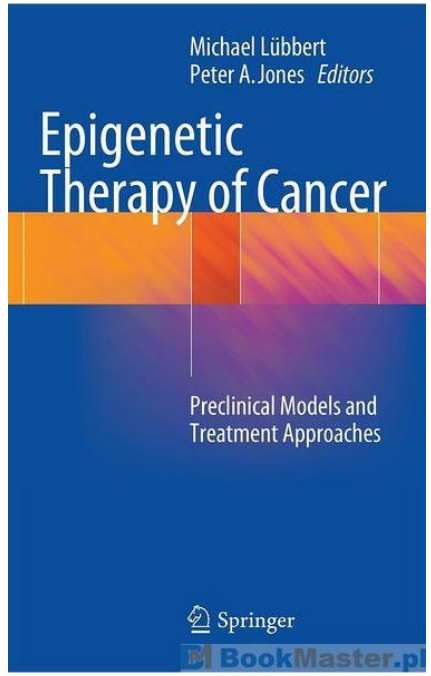
- cancer
- neurodevelopmental
- psychiatric
- imprinting disorders
- autoimmune
- cardiopulmonary
- aging
- obesity



DNA methylation is targetable, i.e. it can be altered, and can be a useful clinical test

Multiple FDA approved cancer drugs modify the epigenome

FDA approved tests for cancer include detection of disease associated methylation patterns



Leading Edge
Review

Cell

Cancer Epigenetics: From Mechanism to Therapy

Mark A. Dawson[1,2] and Tony Kouzarides[1,*]
[1]Gurdon Institute and Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, UK
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The epigenome and DNA methylation show environmental susceptibility in humans

- Diet
- Metals
- Infection
- Air pollution
- Endocrine disruptors
- Childhood SES
- 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)

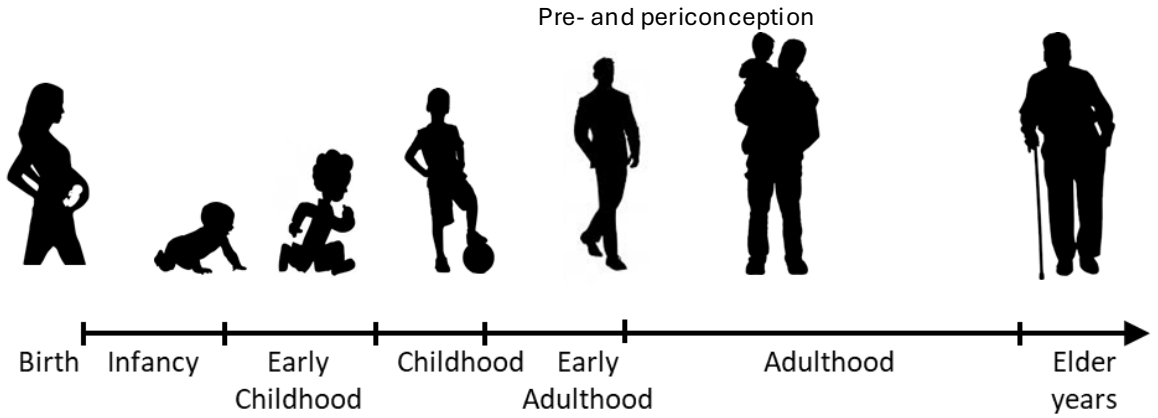
(Bakulski et al, Environmental and Molecular Mutagenesis 2014)

How can we use DNA methylation measures in ECHO to improve children's health?

What are our research goals?

Overarching Goal: Improve Child Health

Health and disease across the lifespan



1: Disease Prevention

- Prevent disease from occurring
- Prevent associated symptoms & disabilities

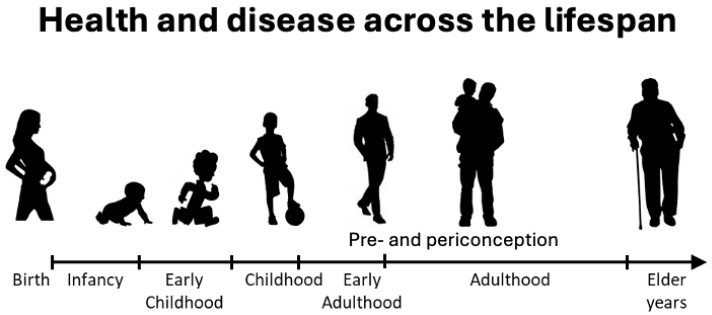
2: Disease Treatment

- Cure affected individuals
- Mitigate associated symptoms & disabilities

3. Disease Prediction

- Diagnosis
- Prognosis
- Response to treatment

How to we address these goals?



Identify causes, mechanisms, risk factors

1: Disease Prevention

- Prevent disease from occurring
- Prevent associated symptoms & disabilities

2: Disease Treatment

- Cure affected individuals
- Mitigate associated symptoms & disabilities

Identify predictive biomarkers

3. Disease Prediction

- Diagnosis
- Prognosis
- Response to treatment

Epigenomic Epidemiology Framework to Address Our Research Goals

Investigate MECHANISMS

Biospecimen type and timing may matter!!

Result | **Goal**

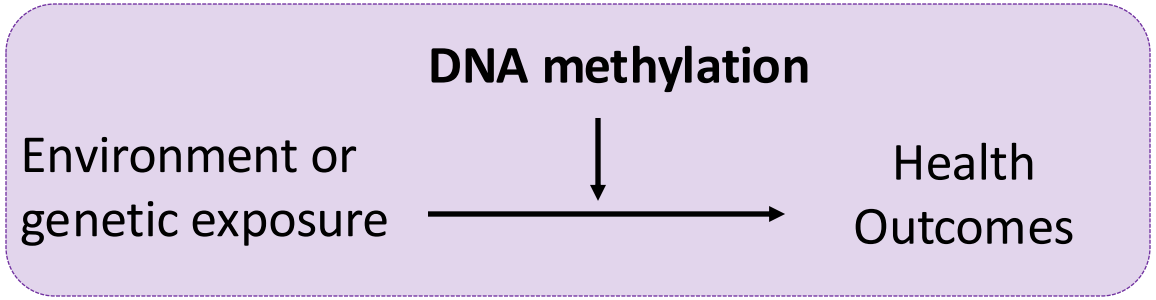
Epigenetic **MEDIATION** of exposure effects on health:

Environment or genetic exposure → **DNA methylation** → Health Outcomes

- Identify actionable biology

Prevention
Treatment

Epigenetic **MODIFICATION** of exposure effects on health:



- Provide risk context
- Identify susceptible subgroups

Prevention
Treatment

(precision public health?)

Epigenomic Epidemiology Framework to Address Our Research Goals

BIOMARKER utility

Surrogate/non-invasive accessible biospecimens okay and preferred!

BIOMARKER of health outcomes**:**

DNA methylation* ⟶ Disease

DNA methylation ⟶ Prognosis or response to treatment

*okay if a consequence of disease for biomarker purposes

Result

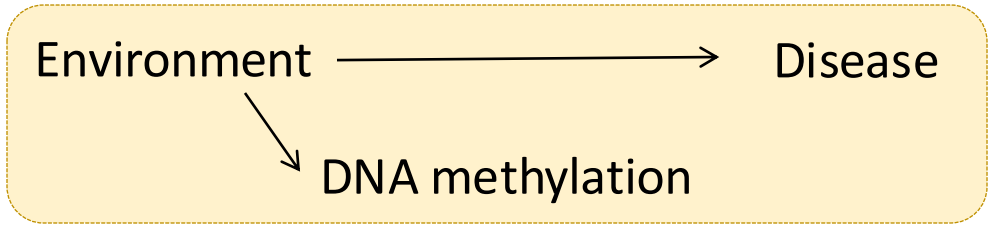
- Accurately predict disease
- Identify clinical marker
- Clinical trial metric

Goal

Improve high risk identification and clinical diagnosis

Effective treatment (precision medicine)

BIOMARKER of environmental exposures:



- Helps to identify modifiable risk factors
- Could monitor effectiveness of interventions

Prevention & Treatment

We can test these hypothesis using different scales or “units” of DNA methylation measures

Global DNA methylation
Reflects broad changes across the genome

Methylation Scores
Composite derived scores, often summing methylation levels at hundreds to thousands of CpGs

Biologic pathways/gene sets
Subset of methylation measures, *a priori* selected based on gene annotations

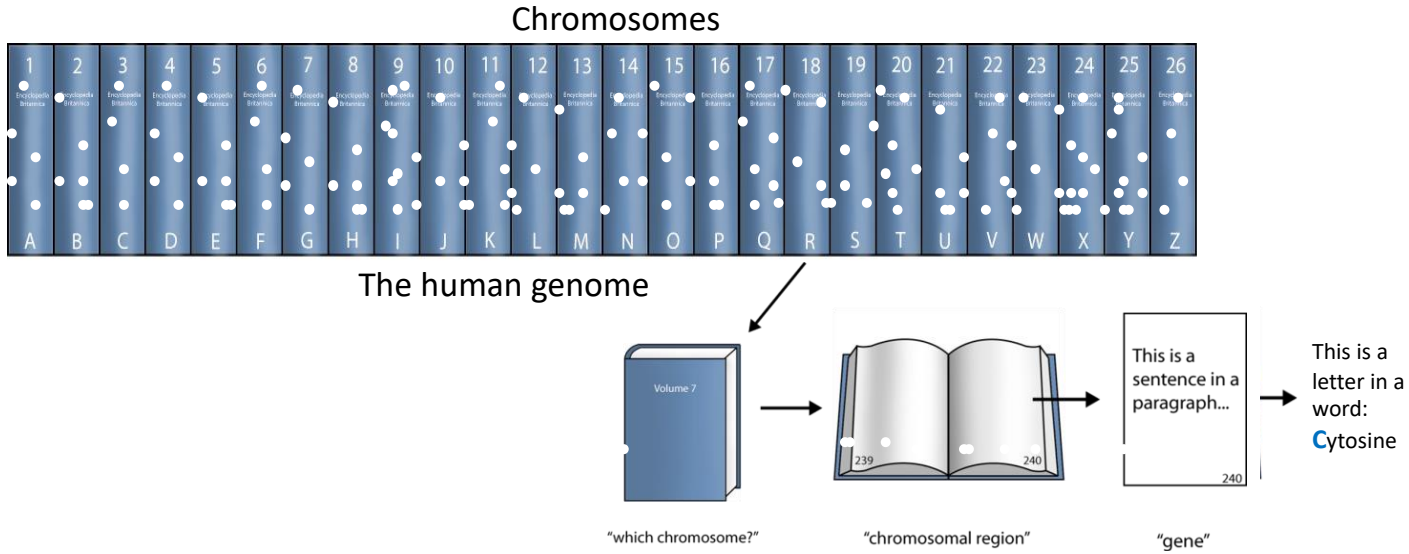
Regional DNA methylation
DNA methylation patterns at a local cluster or group of CpGs

Individual CpG
One single physical position in the genome

largest

Amount of DNA methylome/ genome covered

smallest



Epigenomic Epidemiology Framework: A Few Empiric Examples

Epigenetic **MEDIATION** of exposure effects on health**:**

Environment or genetic exposure → **DNA methylation** → Health Outcomes

EDITOR'S CHOICE

Mediation by Placental DNA Methylation of the Association of Prenatal Maternal Smoking and Birth Weight FREE

Andres Cardenas ✉, Sharon M Lutz, Todd M Everson, Patrice Perron, Luigi Bouchard, Marie-France Hivert

American Jou

https://doi.or

Published: 0

SCIENCE ADVANCES | RESEARCH ARTICLE

HUMAN GENETICS

DNA methylation as a
between prenatal adve
metabolic disease in ac

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Kate M. Xu,[3,5] Biobank-based Integrative
Erik W. van Zwet,[3] L. H. Lumey,[1,6†] Basti

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Nat Commun. ; 6: 6304. doi:10.1038/ncomms7304.

Genome-wide Association Study Identifies P
Specific Loci and Evidence of Epigenetic Mediation in U.S.
Children

Xiumei Hong[1,‡], Ke Hao[2,‡], Christine Ladd-Acosta[3,‡], Kasper D Hansen[4], Hui-Ju Tsai[5,6,7], Xin Liu[5,8], Xin Xu[9], Timothy A. Thornton[10], Deanna Caruso[1], Corinne A Keet[3,11], Yifei Sun[4], Guoying Wang[1], Wei Luo[2,12], Rajesh Kumar[13], Ramsay Fuleihan[13], Anne Marie Singh[14], Jennifer S Kim[13,15], Rachel E Story[13,16], Ruchi S Gupta[5], Peisong Gao[17], Zhu Chen[1], Sheila O. Walker[1], Tami R Bartell[5], Terri H Beaty[3], M Daniele Fallin[18], Robert Schleimer[19], Patrick G Holt[20], Kari Christine Nadeau[21], Robert A Wood[11], Jacqueline A Pongracic[13], Daniel E Weeks[22], and Xiaobin Wang[1,23,*]

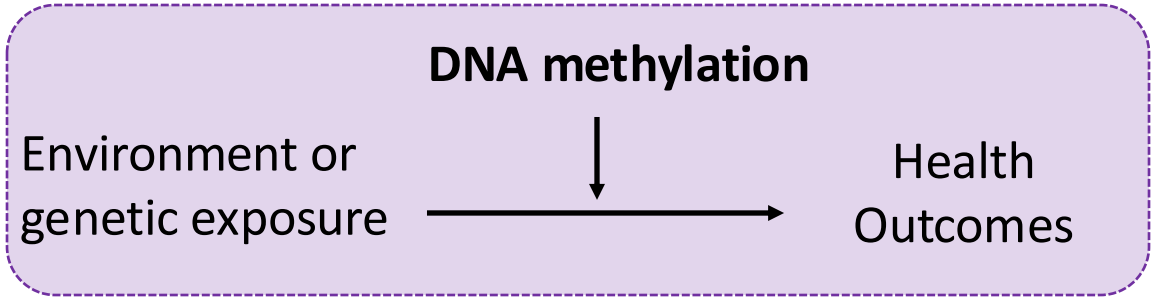
<u>Methylation Mediation Analyses Align with ECHO Scientific Priority #1:</u> Identify early exposures considering multiple exposure effects, multiple health outcomes impacted, and advanced **methods and study designs to identify novel causal relationships***

**Note: causal inference testing tools available (e.g. Mendelian Randomization)*

Epigenomic Epidemiology Framework: A Few Empiric Examples

Few examples exist…..more on this later…opportunities!

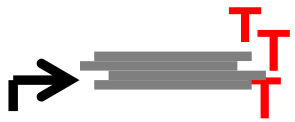
Epigenetic **MODIFICATION** of exposure effects on health**:**



Unaffected Individual

GCAAC…CGTA…

GCTAC…^{m}CGTA…



Mutant protein product

Affected Individual

GCAAC….^{m}CGTA…

GCTAC…. CGTA…

<u>Methylation Modification Analyses Align with ECHO Scientific Priority #3:</u>

Identify factors that **buffer or amplify the effects of** exposures or genetic susceptibility*

This could also provide a mechanism for gene-environment interaction!

Epigenomic Epidemiology Framework: A Few Empiric Examples

Few examples exist for pediatric populations…..more on this later…opportunities!

BIOMARKER of health outcomes:

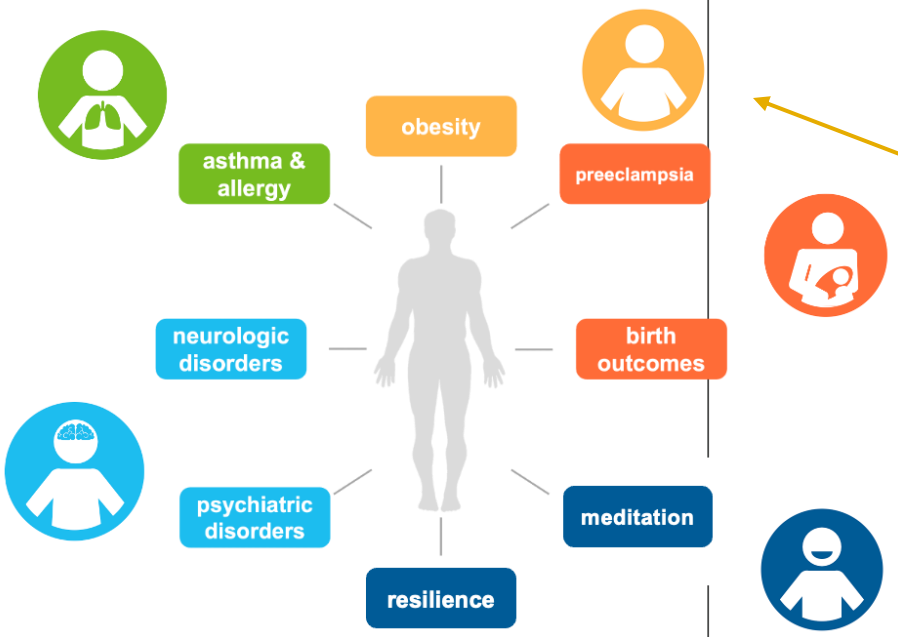
DNA methylation* ⟶ Disease

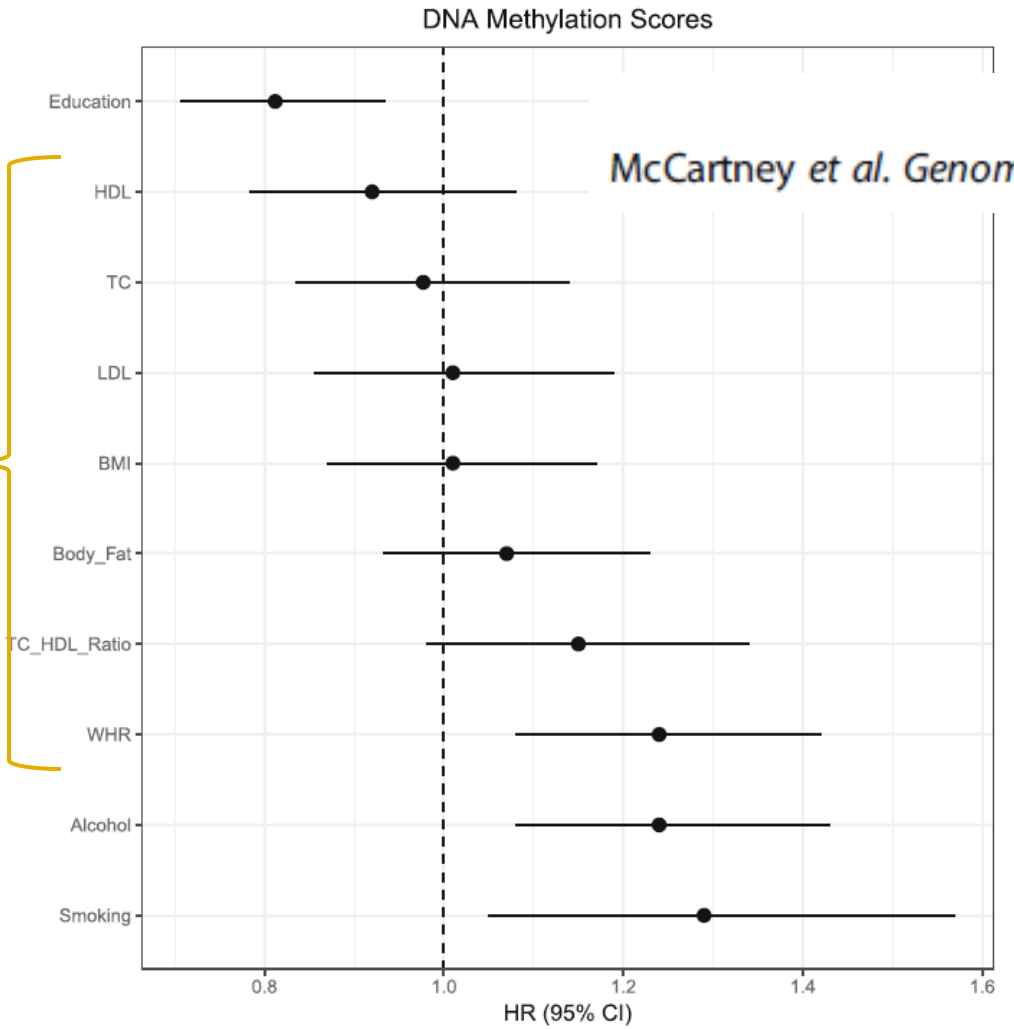
DNA methylation ⟶ Prognosis or response to treatment

*okay if a consequence of disease for biomarker purposes

DNA methylation Classifiers Of Exposures **And Outcomes**

what about in pediatric population?





McCartney *et al. Genome Biology* (2018) 19:136

Fig. 3 HRs for epigenetic (DNAm) predictors of mortality. *Forest plots* show HRs for DNAm scores for health and lifestyle factors. Effect sizes are per standard deviation with the exception of phenotypic smoking, for which never smokers are used as a reference group. *Horizontal lines* represent 95% CIs

FDA Approved (marketplace) DNA Methylation Based Tests for **Cancer Diagnosis**

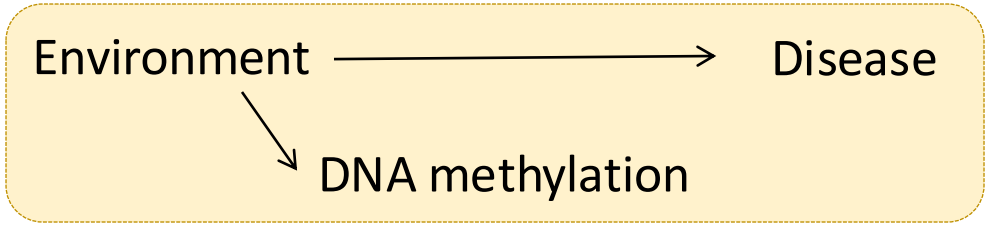
	OC-SENSOR®	ColoGuard®	Epi proColon®	Epi proColon®	IKZF1/BCAT1
Assay	Fecal immunochemical test (FIT); 100 μg Hb/g	*KRAS* mutations, methylated *NDRG4*, *BMP3* and hemoglobin	Methylated *SEPT9*	Methylated *SEPT9*	Methylated *BCAT1* and/or *IKZF1*
Biosample	Stool	Stool	Blood	Blood	Blood
Study cohort size	9989 (65 tumors)	9989 (65 tumors)	1544 (44 tumors)#	1510 (53 tumors)#	2101 (85 tumors)
Specificity	94.9%	86.6%	80.0%	91.5%	93.8%
Sensitivity	73.8%	92.3%	68.2%	48.2%	65.9%
Advanced precancerous lesions*	23.8%	42.4%	21.6%	11.2%	6.2%
Stage I	65.5%	89.7%	41.1%	35.0%	37.9%
Stage II	76.2%	100.0%	83.3%	63.0%	69.0%
Stage III	90.0%	90.0%	80.0%	46.0%	72.5%
Stage IV	75.0%	75.0%	100.0%	77.4%	93.8%
Data reference	(Imperiale et al., 2014)	(Imperiale et al., 2014)	(Potter et al., 2014)	(Church et al., 2014)	(Pedersen et al., 2015b)

**Defined as advanced adenomas and sessile serrated polyps measuring 1 cm or more*
#Standardized estimates

<u>Methylation Biomarkers of Pregnancy and Child Health Outcomes Align with ECHO Scientific Priority #2:</u>
Identify health outcome trajectories and **related endophenotypes**. These can be used to inform diagnosis, prognosis, treatment plans and/or identify high risk subgroups for precision screening/follow up

Epigenomic Epidemiology Framework: A Few Empiric Examples

BIOMARKER of environmental exposures:



DNA methylation patterns in children reflect prenatal smoking exposure. These patterns are distinct from second hand and personal smoking and "DNAm scores" developed to measure prenatal smoking exposure using offspring blood through mid-adulthood

EC0533 (Shorey-Kendrick): Is prenatal smoking exposure associated with childhood blood pressure?

- Examined this question using traditional exposure ascertainment method and 2 empiric biomarkers - cotinine and DNA methylation prenatal smoking score

Lyndsey Shorey-Kendrick

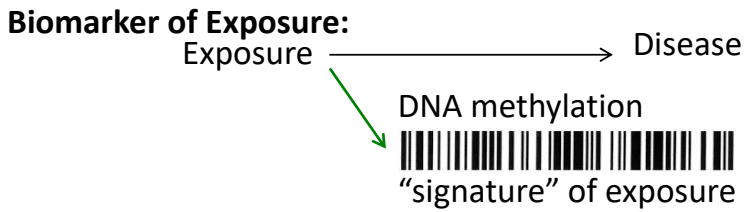
Similar Results were Observed Across Prenatal Smoking Exposure Measures

Association between prenatal smoking exposure and childhood blood pressure (BP)

	Systolic BP (Z-score)		**Diastolic BP (Z-score)**	
	Effect estimate (95% CI)	***P* value**	**Effect estimate (95% CI)**	***P* value**
Any prenatal tobacco exposure (n=13,120 systolic BP; 13,083 diastolic BP)				
Categorical self-report: yes/no				
Crude	0.034 (-0.014, 0.082)	0.162	0.220 (0.18, 0.26)	**<0.001**
Adjusted*	0.021 (-0.025, 0.069)	0.388	0.196 (0.155, 0.237)	**<0.001**
Pregnancy urine cotinine (n=2763 systolic BP; 2726 diastolic BP)				
Continuous log(cotinine)				
Crude	0.013 (0.007, 0.019)	**<0.001**	0.038 (0.033, 0.043)	**<0.001**
Adjusted*	0.016 (0.009, 0.023)	**<0.001**	0.033 (0.028, 0.039)	**<0.001**
Categorical cotinine: Active vs no exposure[37]				
Crude	0.251 (0.16, 0.34)	**<0.001**	0.489 (0.416, 0.562)	**<0.001**
Adjusted*	0.215 (0.122, 0.308)	**<0.001**	0.417 (0.341, 0.492)	**<0.001**
DNAm PrenatalSmokeScore (n=971)				
Continuous methylation scores				
Crude	0.436 (0.15, 0.722)	**0.003**	0.209 (-0.023, 0.441)	*0.079*
Adjusted*	0.302 (0.011, 0.593)	**0.042**	0.093 (-0.145, 0.332)	0.443
Categorical methylation: active versus no exposure				
Crude	0.190 (0.077, 0.303)	**0.001**	0.103 (0.011, 0.195)	**0.027**
Adjusted*	0.142 (0.029, 0.255)	**0.014**	0.065 (-0.027, 0.157)	0.167

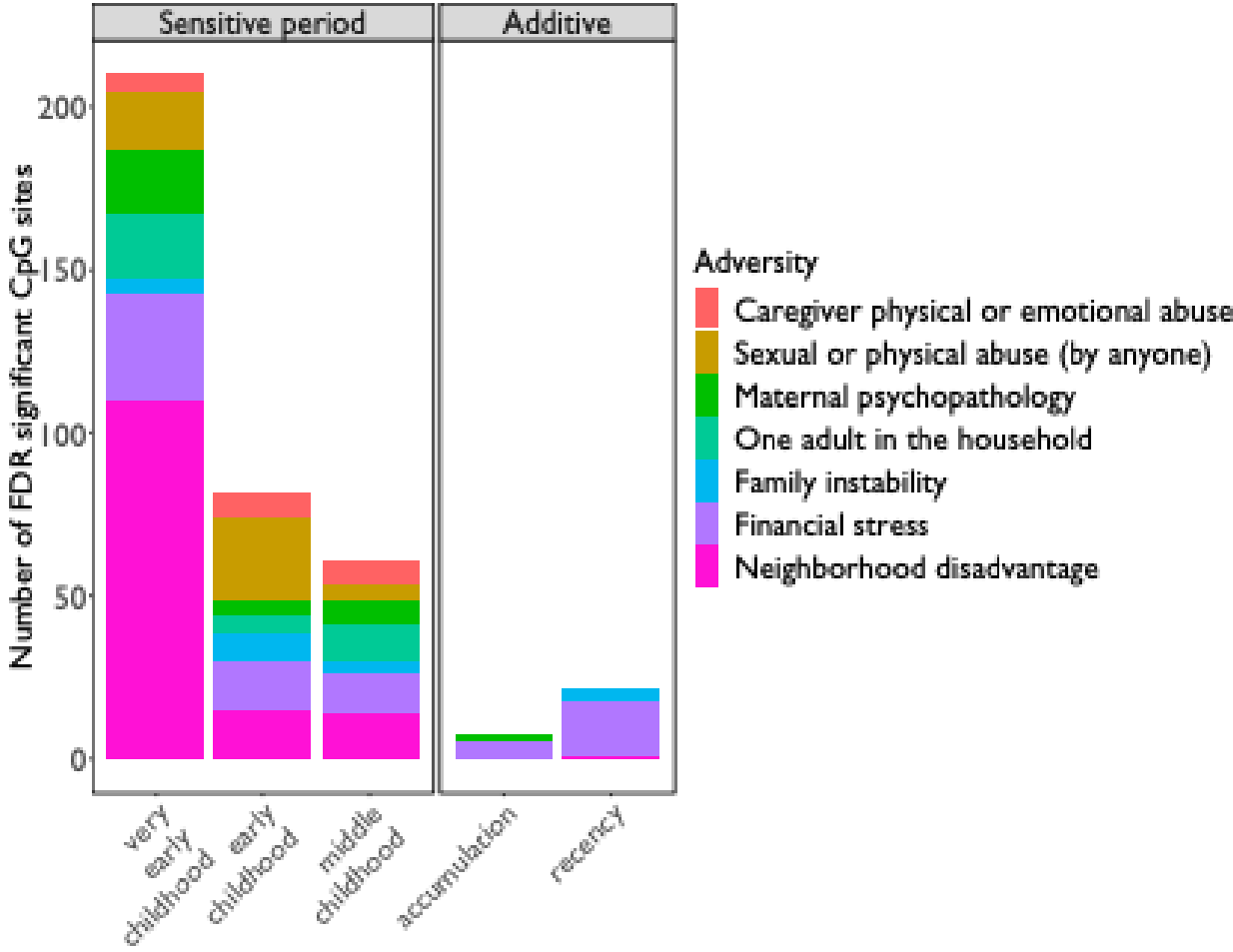
BP indicates blood pressure. Boldface indicates a p-value below a 0.05 significance threshold.

Provides proof of principle evidence and implications



- Methylation profiles may provide complementary empirical biomarkers of exposure, addressing potential misclassification
- May help to identify more difficult to measure exposures and critical windows of exposure
- May capture inter-individual differences in biologic response to exposures ("real world" multiple exposures?)
- Easily accessible (surrogate) tissues okay

Child methylation patterns have been shown to reflect specific periods of exposure



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

DNA methylation exposure biomarkers support multiple ECHO 2 Scientific Priorities

Note: not interested in mechanism here, using it as an exposure measurement tool!

#1: **Identify early exposures** that impact child health considering multiple exposure effects, multiple outcomes, and use novel methods

#4: **Identify critical windows of exposure impacts on child health**

#5 (MAYBE?): Measure effects of natural events or policy changes on child health outcomes

Challenges and Reflections on ECHO DNA methylation analyses

= Future Opportunities

Challenge/Reflection 1: Sample Size

Extant CHILD DNA methylation data in ECHO

	Measurement Array Name				
Biospecimen Source	450K	EPIC	27K	A&A	**TOTAL**
Dried blood spot	263	448	0	0	**711**
Peripheral Blood	307	46	0	0	**353**
Cord Blood	1195	586	159	244	**2184**
Placenta	61	521	0	85	**667**
Buccal	0	552	0	180	**732**
PBMC	150	140	0	0	**290**
CBMC	0	142	0	0	**142**
Nasal Epithelial	0	0	0	1421	**1421**
Nasal Lavage	0	0	0	907	**907**

DBS = dried blood spot; PBMC = peripheral blood mononuclear cell;
CBMC = cord blood mononuclear cell

Extant MATERNAL DNAm data (n=595 participants)

	Trimester 1	Trimester 2	Trimester 3	TOTAL
Blood	496	300	173	**969**

Challenges = Opportunities

Opportunity: New CHILD Data

Infinium MethylationEPIC v2.0 Kit
Robust methylation profiling microarray providing extensive coverage of CpG islands, genes, and enhancers. Ideal for genetic and rare disease research, cancer research, and classification.

930,000 methylation markers measured per sample

1) Longitudinal methylation measures, mostly blood

ECHO received new measurement data from **885 unique participants (2,206 samples)** with at least 2 methylation measures, from birth though age 21

Data cleaning and file preparation underway, to be released in summer 2025

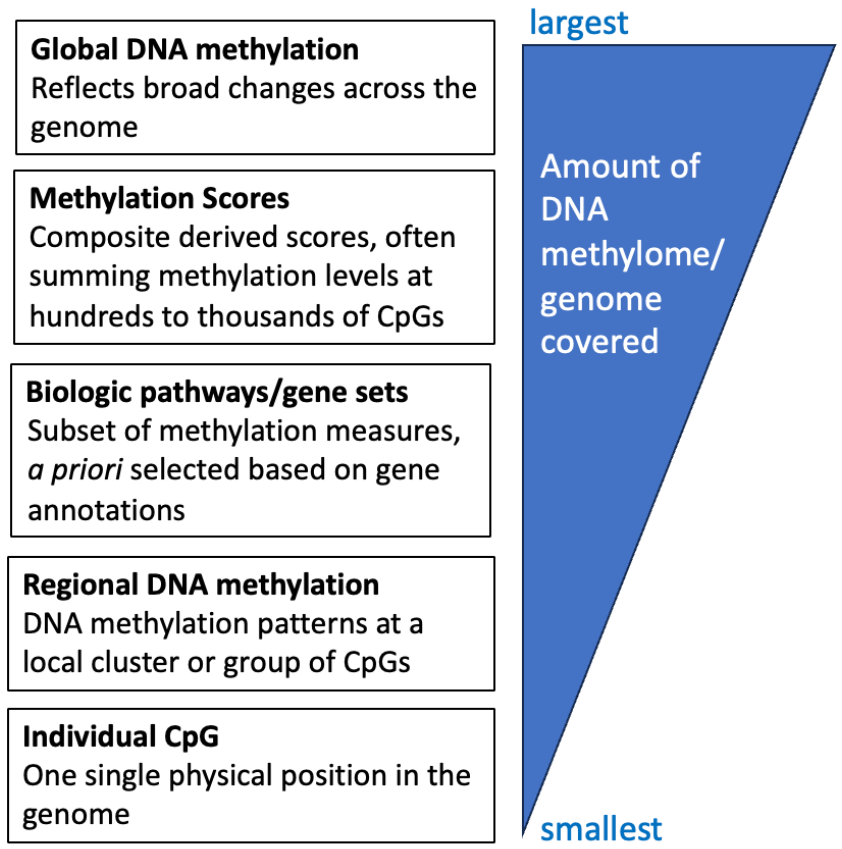
2) Single timepoint methylation measures, blood & saliva

11,781 ECHO child participants with genotyping measures were also sent for DNA methylation measures

Laboratory measurements underway

Challenge/Reflection 2: Most analyses have focused on prenatal environment-methylation associations

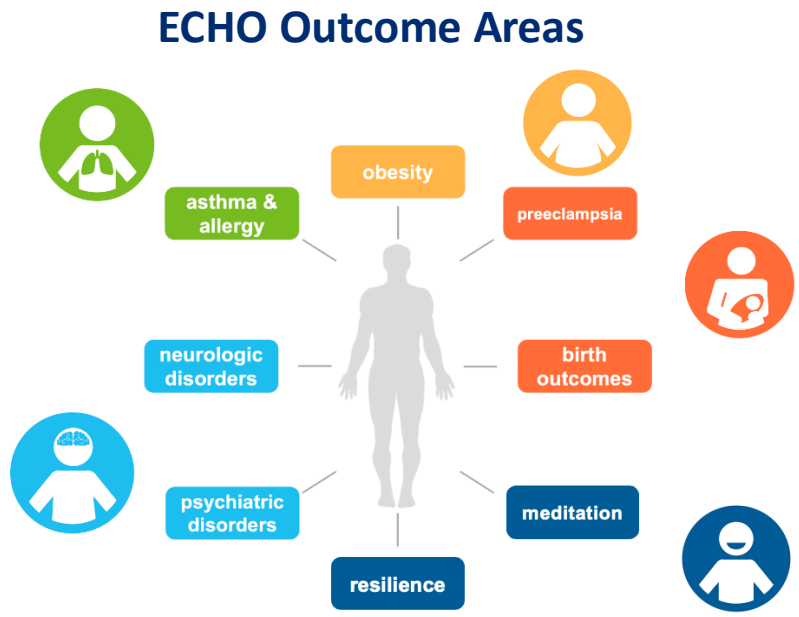
Environmental exposure ⟶ DNA methylation (birth/infancy)



Opportunity: Examine health outcomes and perform mediation testing

Environmental exposure ⟶ DNA methylation ⟶ Child Health

- consider dimensional or multi-outcomes, e.g. quantitative traits
- consider multi-exposures and potential "buffers" beyond more traditional "treatment/pharmacologic" interventions (e.g. behavioral, diet, etc)
- novel/modern methods for causal inference testing, e.g. Mendelian randomization and methods that take reverse causality and correlation into account



Opportunity: Examine DNA methylation as biomarker for diagnosis, intervention or trial effectiveness, response to treatment (precision medicine): in childhood

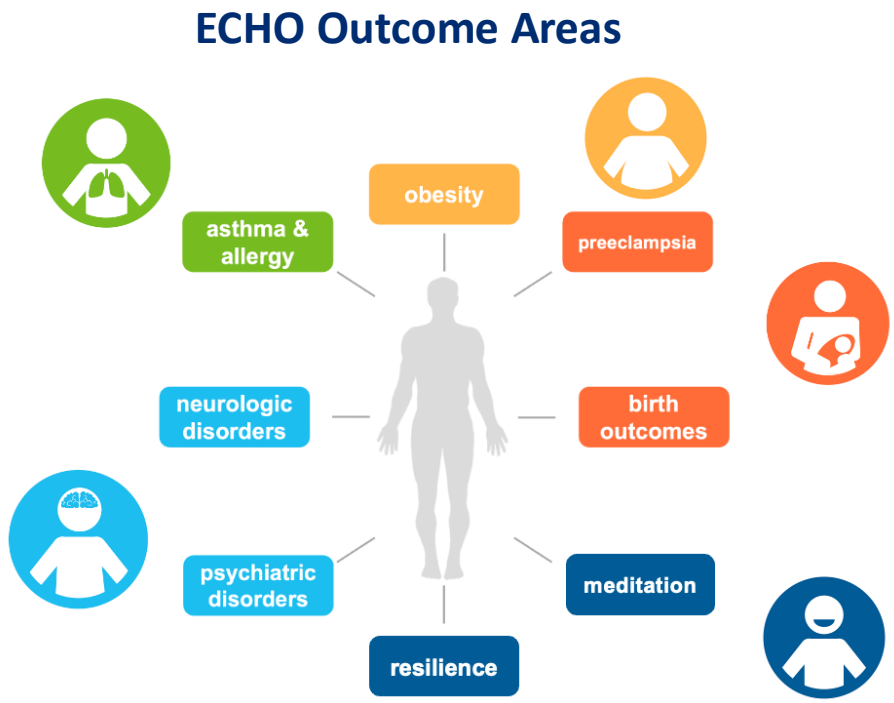
DNA methylation ⟶ Disease

DNA methylation ⟶ Prognosis or response to treatment

Clinical Trials (ECHO IDeA States Pediatric Clinical Trials Network)

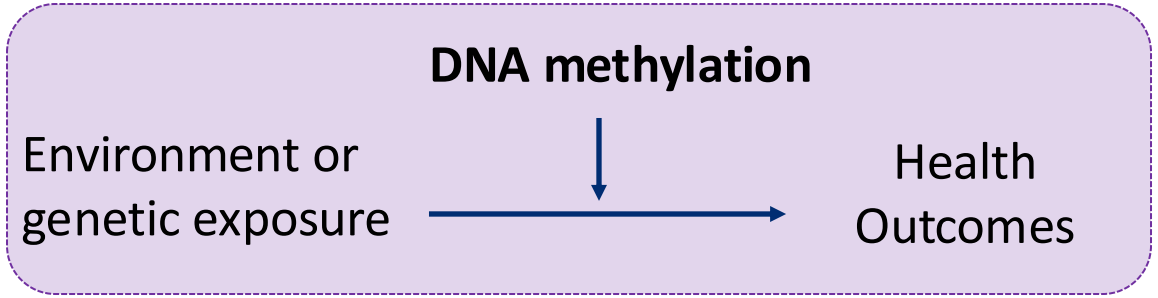
IDeA States Pediatric Network
ECHO A program supported by the NIH

Infants and children living in rural areas are less likely than those living elsewhere to have a chance to enroll in clinical research, especially clinical trials.



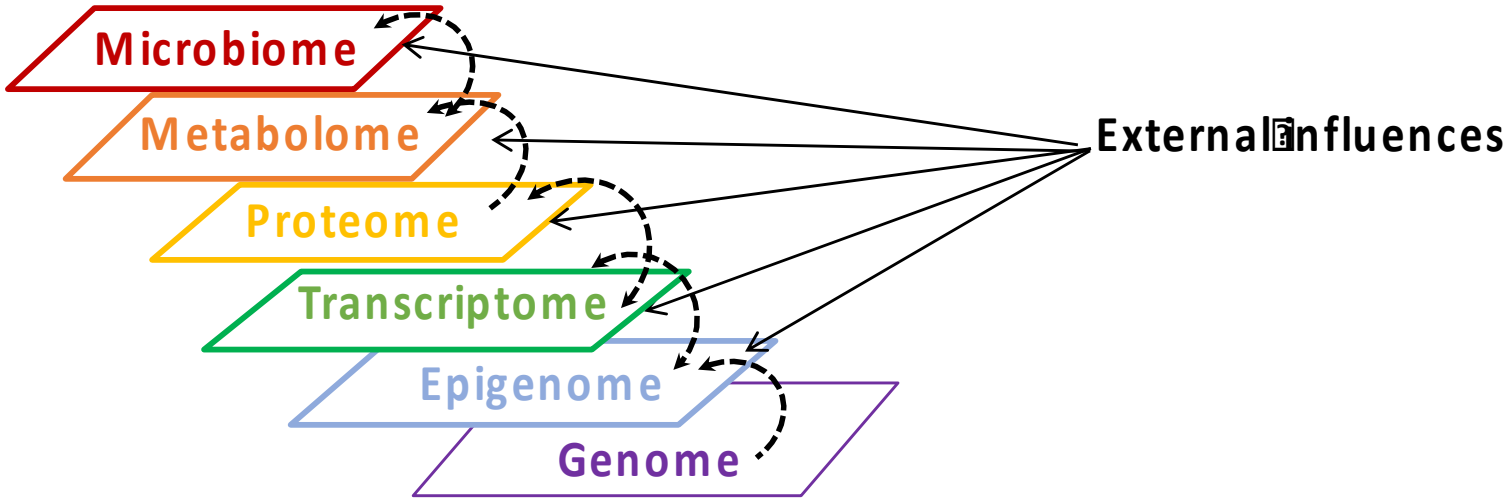
Challenge/Reflection 4:

Epigenetic **MODIFICATION** of exposure effects on health:



Opportunity 3: Test for epigenetic modification (aligned with scientific priority #3 and precision public health?)

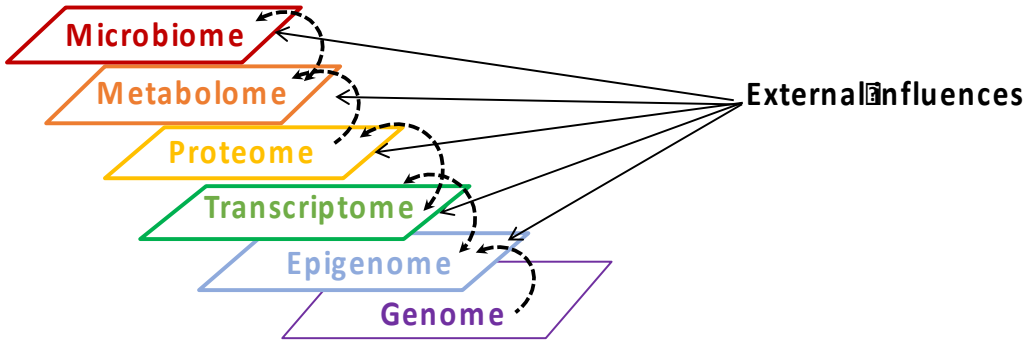
Challenge/Reflection 5: Lack of unified data across “omics” limits integration



Multi-omic analyses

- Data availability
- Lack of “true integration” across –omics

Opportunity: New unified genomic and DNA methylation data available in 2025



Infinium MethylationEPIC v2.0 Kit
Robust methylation profiling microarray providing extensive coverage of CpG islands, genes, and enhancers. Ideal for genetic and rare disease research, cancer research, and classification.

930,000 methylation markers measured per sample

- Can inform mechanisms
- Provide methodologic tools
- Perhaps in combination improve predictive biomarkers

  - Can machine learning/artificial intelligence be useful here?

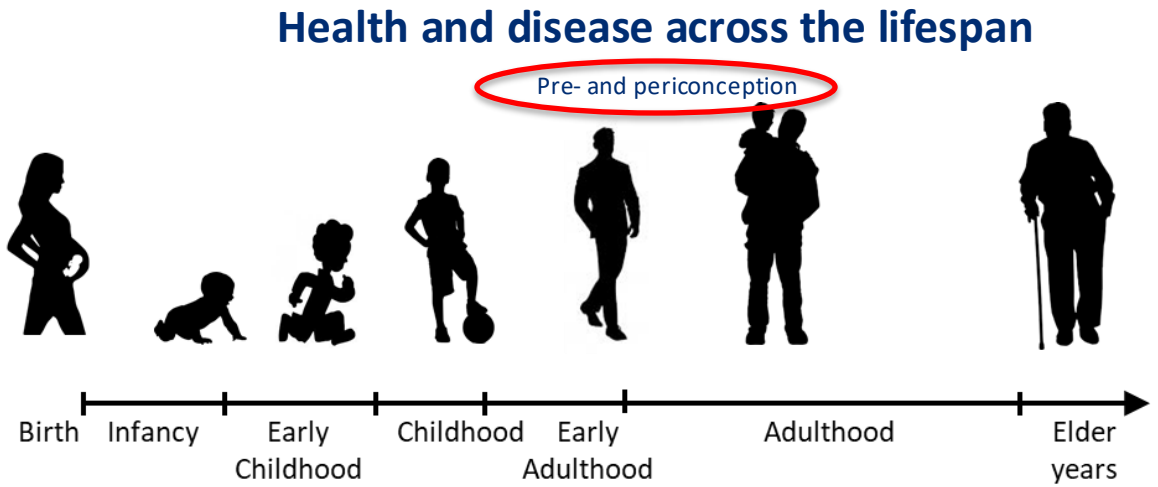
New CHILD methylation measures from blood & saliva (n=11,781) **ALSO HAVE GENOTYPING,** subset may have microbiome, metabolome, other "ome" but need to consider timing

Challenge/Reflection 6: How is ECHO uniquely positioned to address significant and impactful unanswered areas of research?

Opportunity: Leverage the Study Design

We have largely focused on the pregnancy and offspring:

- What impact do maternal methylation cues have?
- What impact do father methylation cues have?
- How do the maternal and paternal cues get "transmitted" to the offspring?
  - direct via sperm/egg?
  - indirect via behaviors?
  - something else?



Opportunity: Leverage the Well-Captured Developmental Life Stage to Assess Developmental Epigenetic Questions

1) Developmental-relevant biospecimen biology: placenta
   - Are the differences in mom or baby placenta sides?
   - Are there differences across tissues in DNA methylation and what do those reflect, e.g. cord blood and placenta

....what other specimens or innovative methods can we use to assess this (e.g. extracellular vesicles, cell free DNA)??

Opportunity: Leverage the Well-Captured Developmental Life Stage to Assess Developmental Epigenetic Questions

2) Given extensive de- and re-methylation and re-programming of primordial germ cells in early in gestation, seems like a critical period:

- Are there methylation differences related to Assisted Reproductive Technology (ART) or other fertility differences?
- What genes and when are they de- and re-methylated
- Others…..

……If we got 2nd pregnancies early enough, we could contribute to this science

Thank you, ECHO!

ECHO

Environmental influences
on Child Health Outcomes

A program supported by the NIH

Please reach out with questions or further discussion:

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