Multi-omic data illuminates the placenta’s role as a mediator between prenatal EDC exposure and preterm birth

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Dept. of Pediatrics, Division of Genetic Medicine | University of Washington
Adjunct: Environmental and Occupational Health Sciences

W INTERDISCIPLINARY CENTER FOR EXPOSURES, DISEASES, GENOMICS AND ENVIRONMENT

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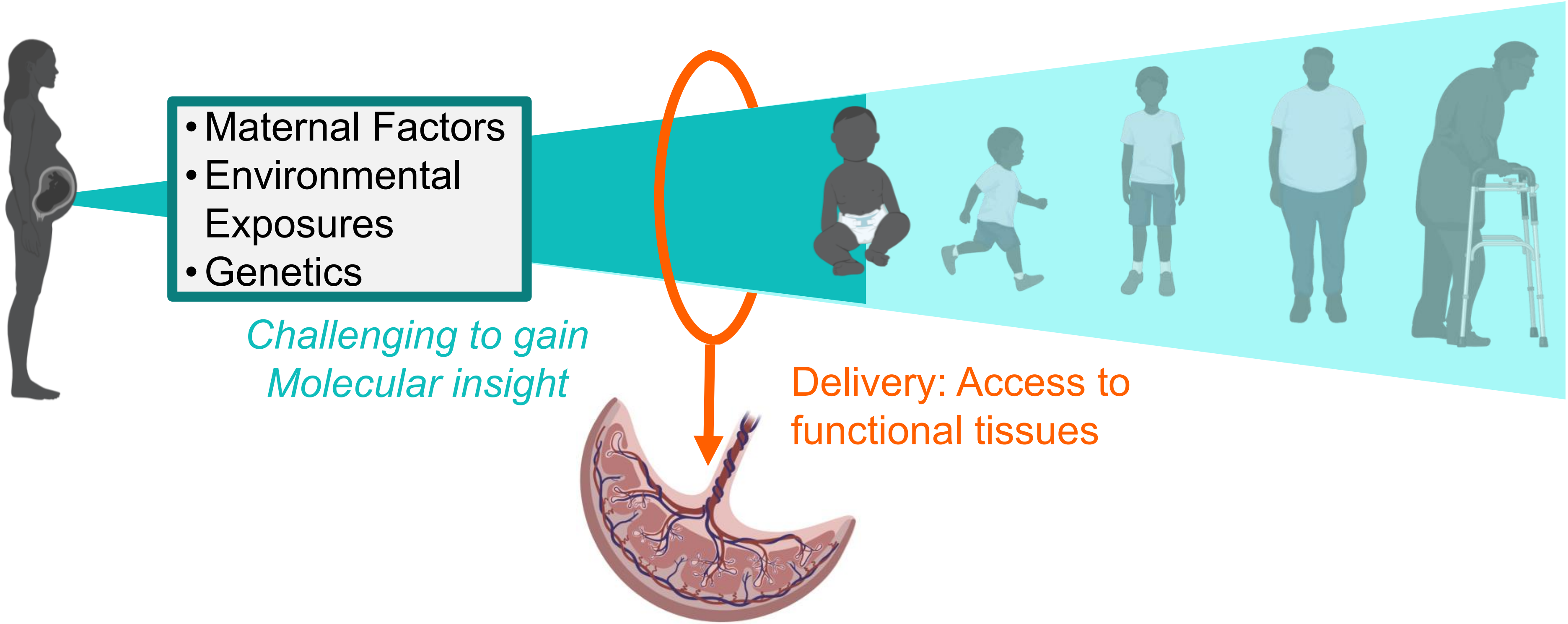
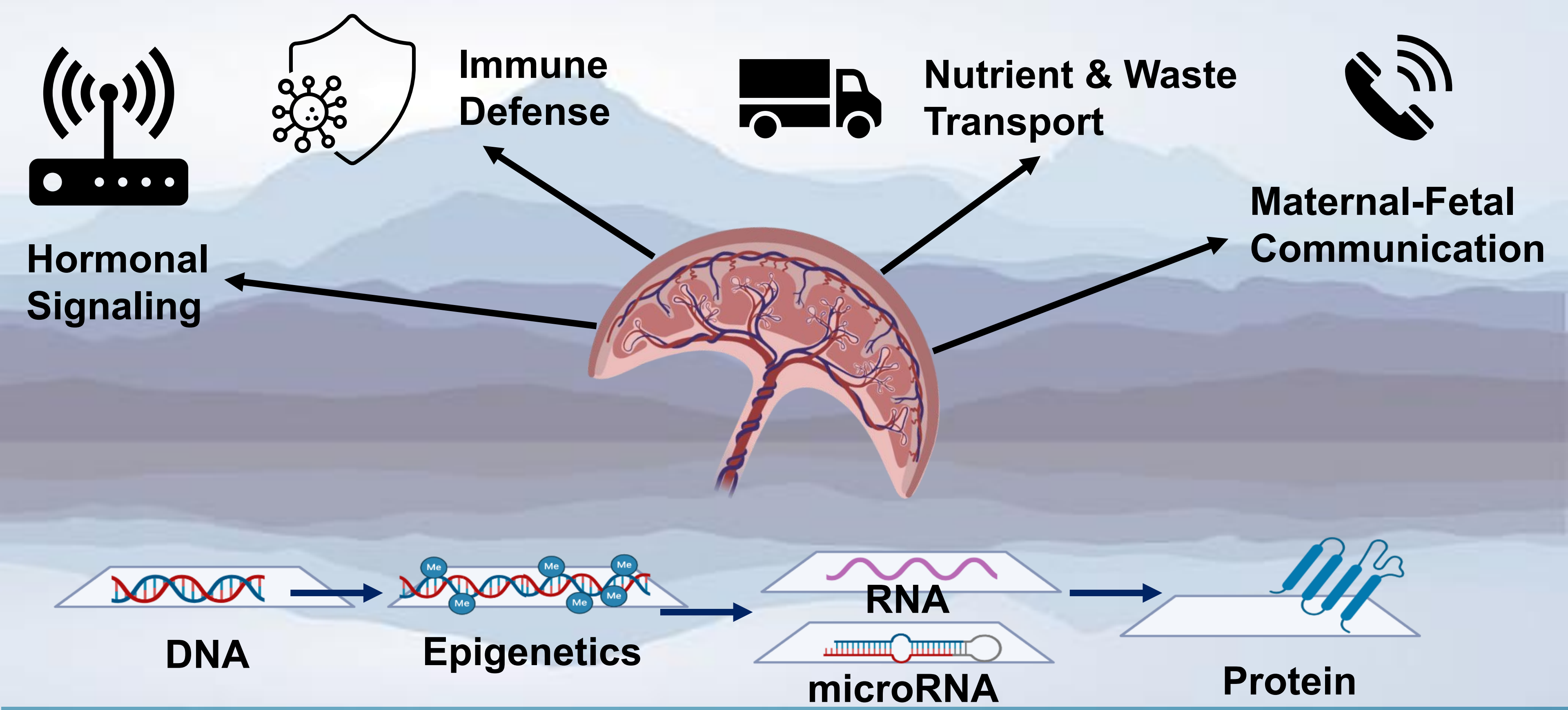
Research Focus: Using Placental omics data to link the Prenatal Environment and Infant Health

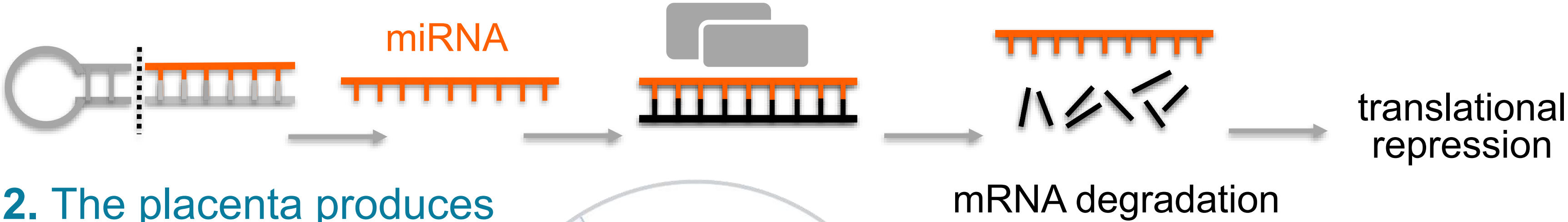


Placental Omics Data: A ***reflection*** of molecular processes essential to placental function

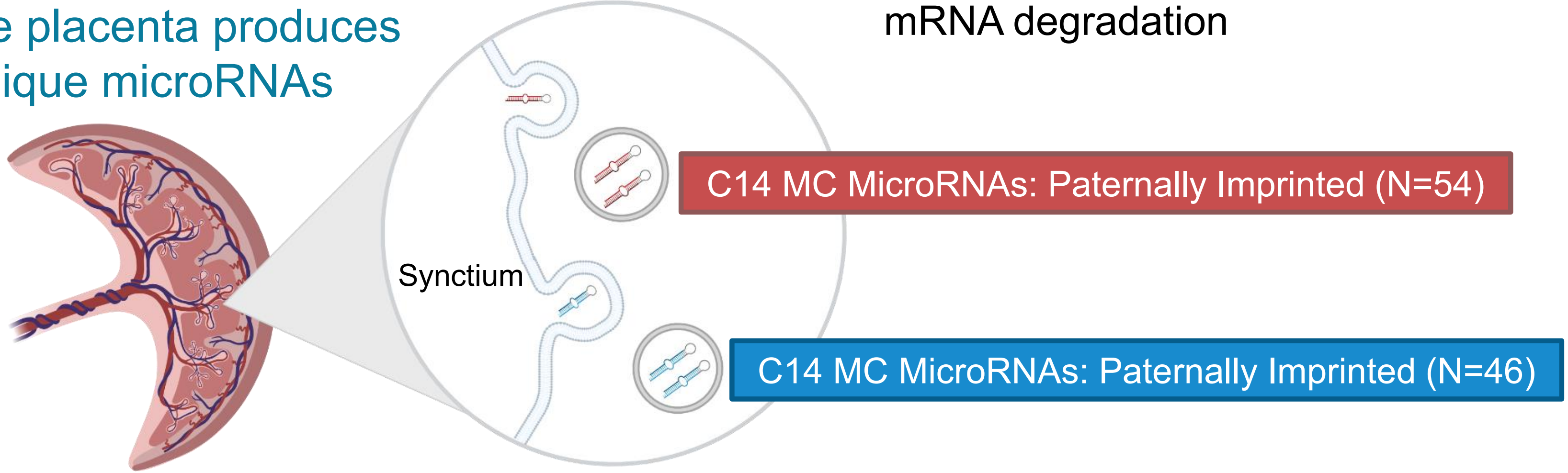


Placental microRNAs are important post transcriptional regulators and ***signaling molecules***

1. microRNAs: Post transcriptional repression of specific gene targets



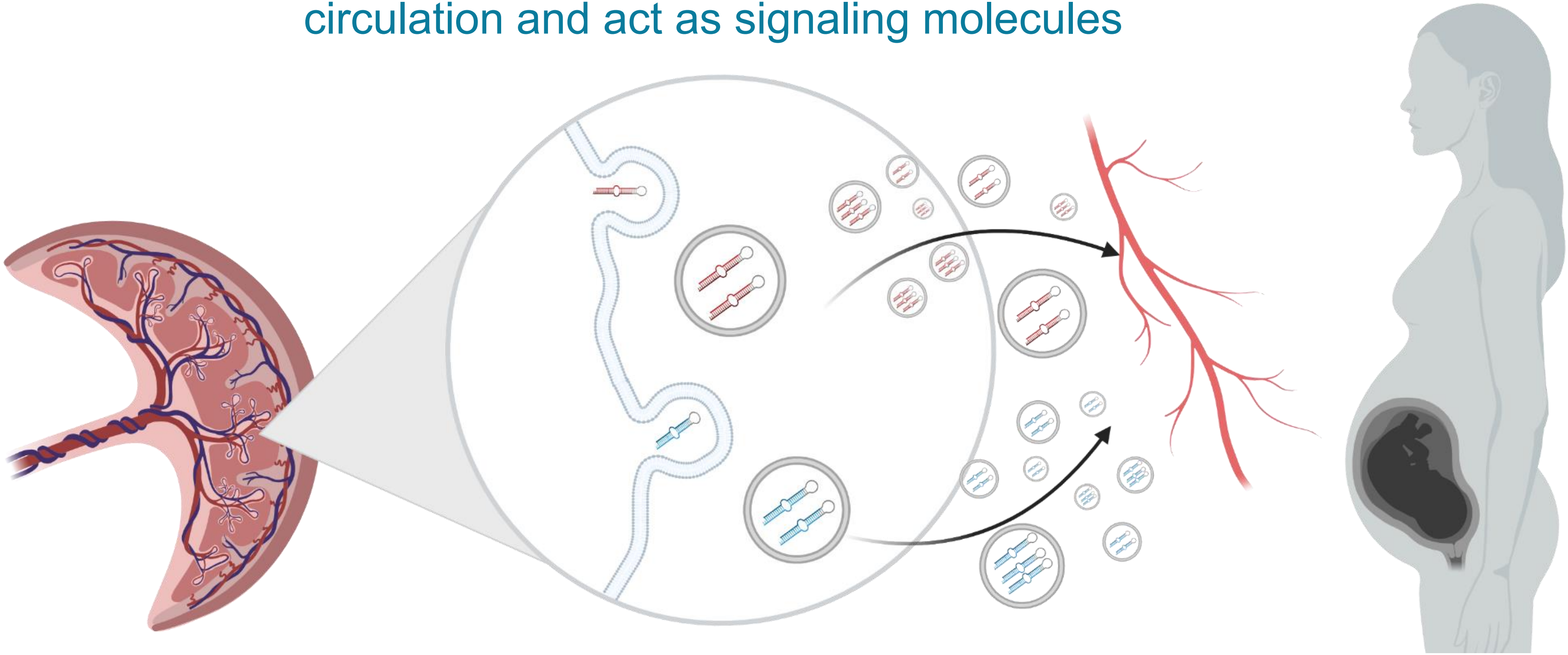
2. The placenta produces unique microRNAs



Mouillet et al, American Journal of Obstetrics and Gynecology 2015

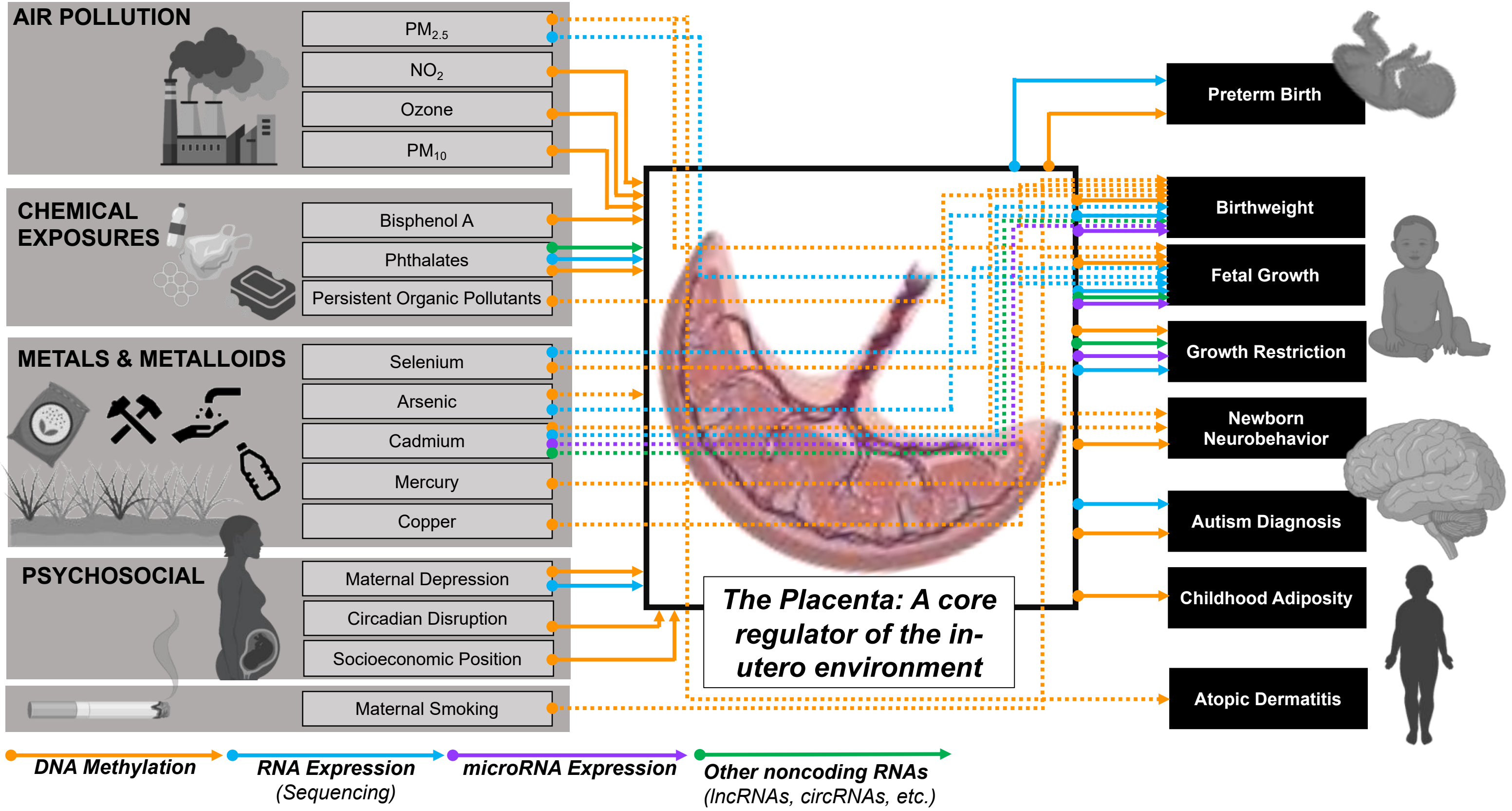
Placental microRNAs are important post transcriptional regulators and *signaling molecules*

3. C14 and C19 Placental microRNAs are released into maternal and fetal circulation and act as signaling molecules



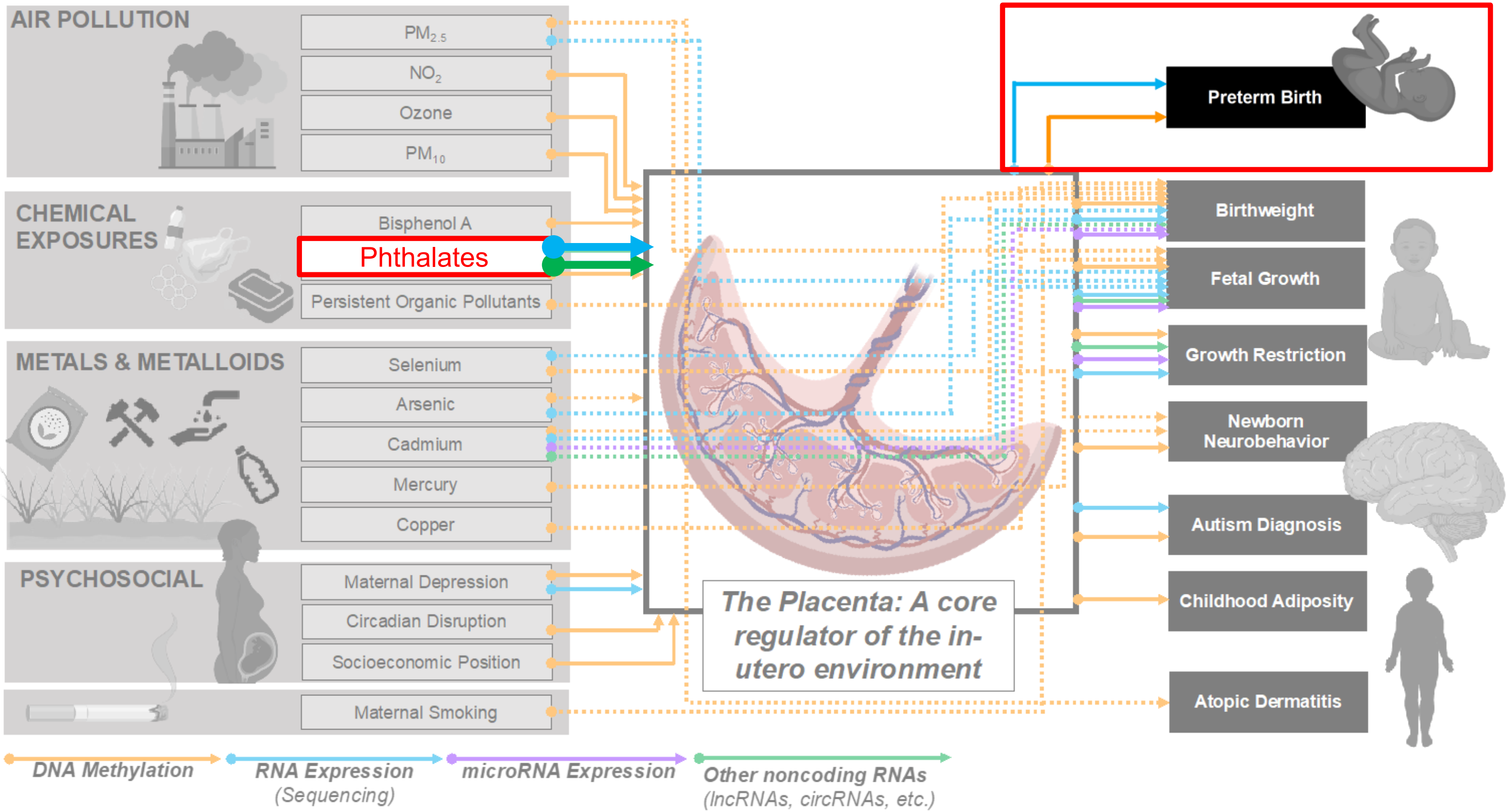
Mouillet et al, American Journal of Obstetrics and Gynecology 2015

Summary of Placental 'Omics Studies 2015-2021



Lapehn and Paquette. Current Environmental Health Reports, 2022

Addressing Research Gaps



Preterm Birth: A Major Healthcare Challenge for Infants and Families

PTB is the leading cause of infant mortality: 35.8% of infant deaths are related to prematurity[1]

Long term morbidity: developmental delays, vision problems, feeding difficulties, cerebral palsy

Preterm Infants Require intensive acute and long term care:

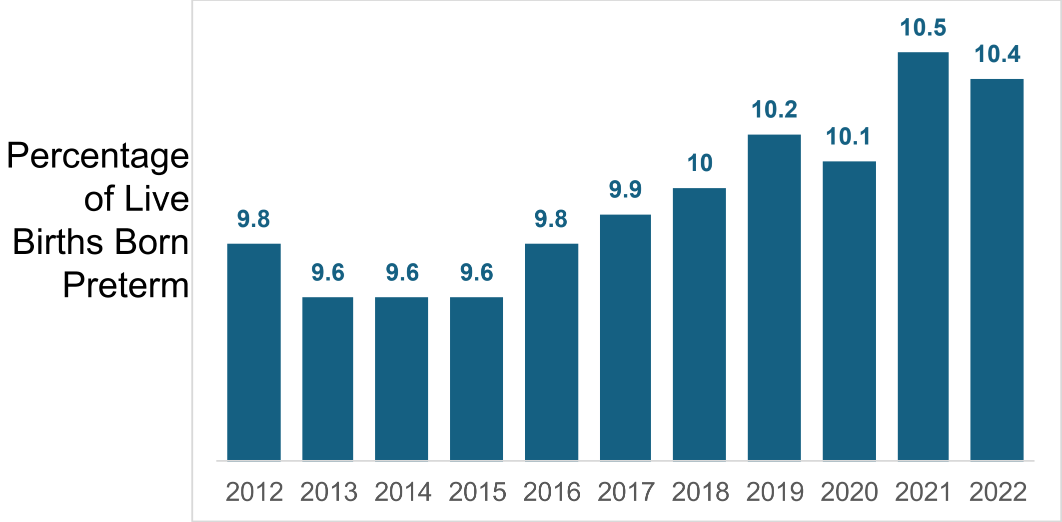
- Daily NICU Cost: 3,500$-20,00$ [2]
- Limited improvements in long term health outcomes in last 2 decades[2]

Annual Societal Cost: $25.2 Billion Dollars (United States)[3]

1.Callaghan WM, et al. Pediatrics. 2006. 2. Muraskas and Parsi, AMA Journal of Ethics, 2008. 3. 2023 March of Dimes report card

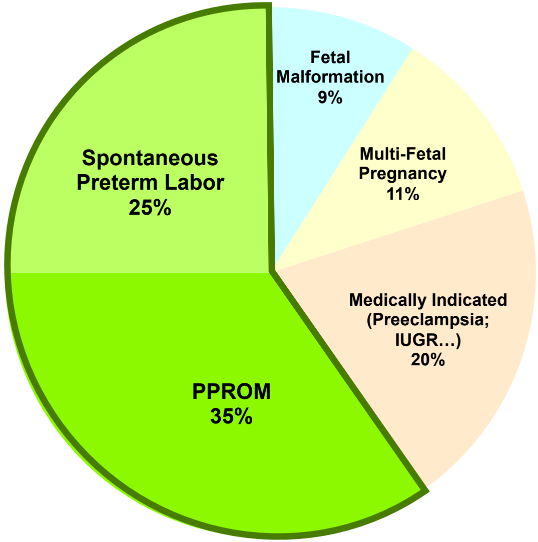
Research Gap: Understanding the Etiology of sPTB

A. Preterm Rate in US (2022): 10.4%



2023 March of Dimes PTB Report Card

B. Causes of Preterm Birth

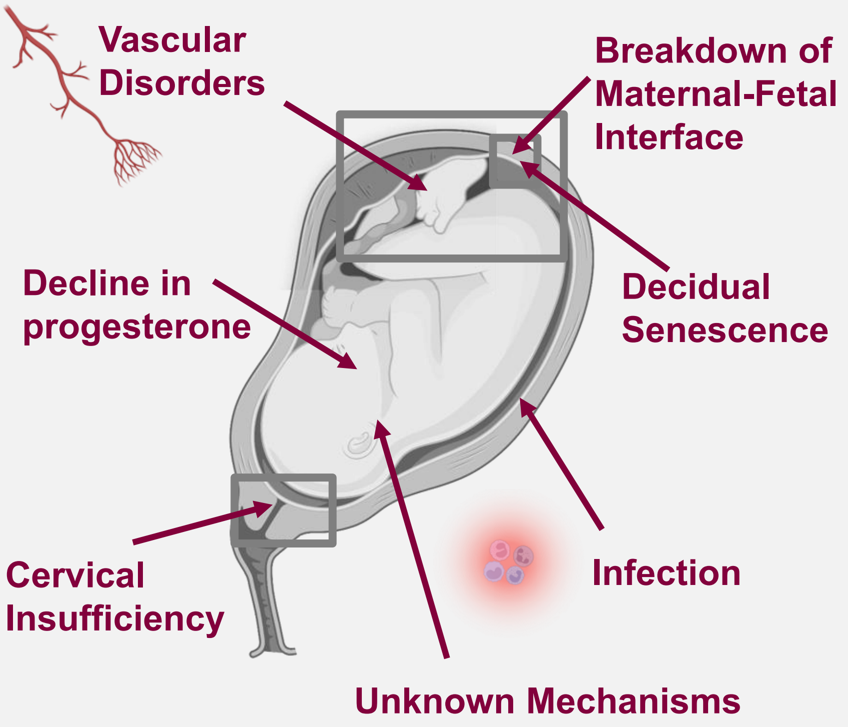


Gracie S et al. BMC Pregn. Childbirth. 2011

Unmet Clinical Needs: Enhanced Diagnostics; Better understanding of causes of spontaneous preterm birth ; Better Prevention Strategies; equitable access to care

Causes & Risk factors for Spontaneous Preterm Labor

A. UNDERLYING MECHANISMS

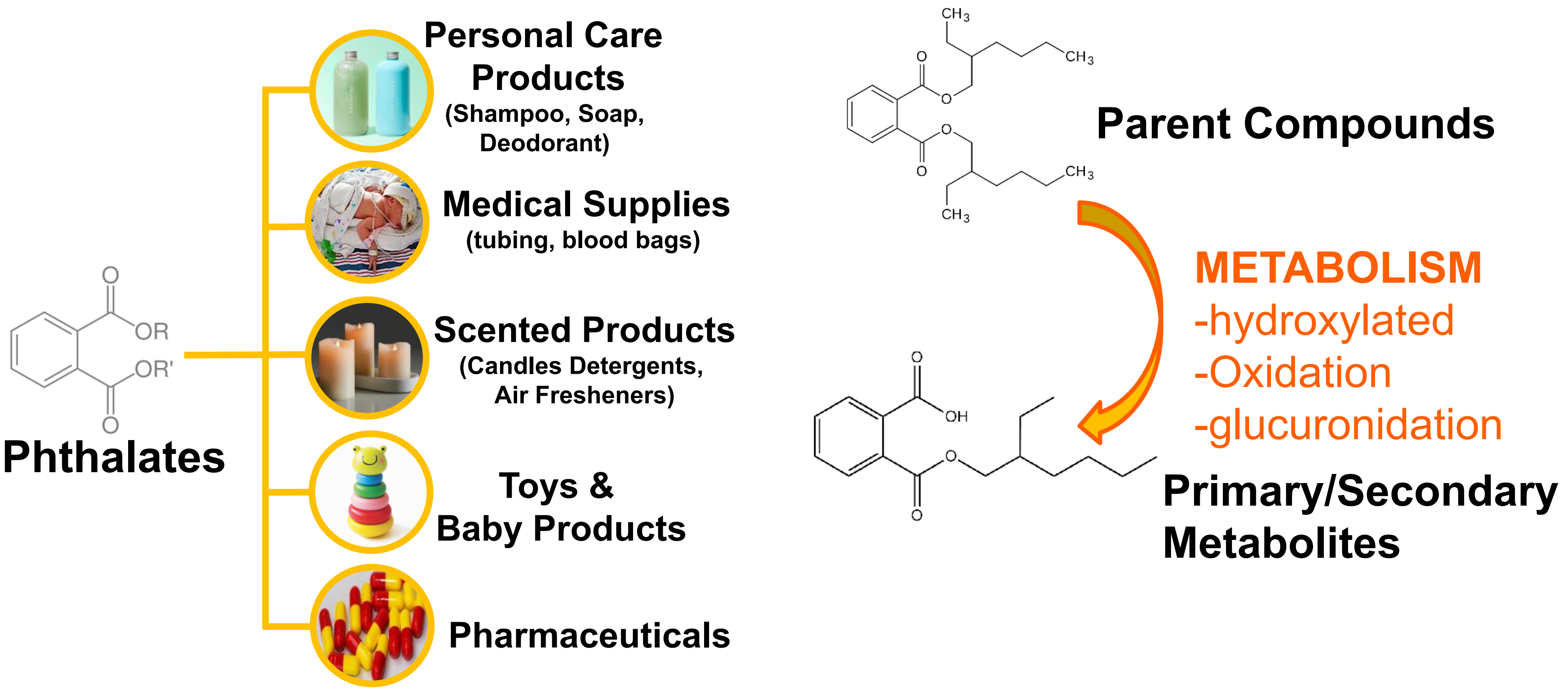


B. RISK FACTORS

Maternal Genetics	• Family History • GWAS 1:N=43,568; 6 genetic loci (Not fetal)[1] • GWAS 2: N=195,555; 7 loci (fetal and maternal)[2] • GWAS 3: N=84,689: 1 locus[3]
Medical Conditions	• Previous History of sPTB • Cervical Insufficiency
Behavioral and sociodemographic Factors	• Lower Education • Geographic Location • Nutritional Deficiencies • Smoking, Illicit Drug use • Maternal Stress
Environmental Exposures	• **Endocrine Disrupting Chemicals** • Air Pollution

1. Zhang et al, NEJM, 2017 2. Nvais et al, Nature Genetics, 2023; 3. Liu et al, Nature Communications, 2019
Adapted from: Roberto Romero et al. Preterm Labor: One Syndrome Many Causes. Science 2014 ; Sheikh et al, Spontaneous Preterm birth and SNPs: A recent Update, BMC Genomics, 2016

Phthalates are Ubiquitous Chemicals present in a variety of consumer goods and products



Prenatal Phthalate Exposure & Increased risk of sPTB

Original Investigation ONLINE FIRST

July 11, 2022

Associations Between Prenatal Urinary Biomarkers of Phthalate Exposure and Preterm Birth

A Pooled Study of 16 US Cohorts

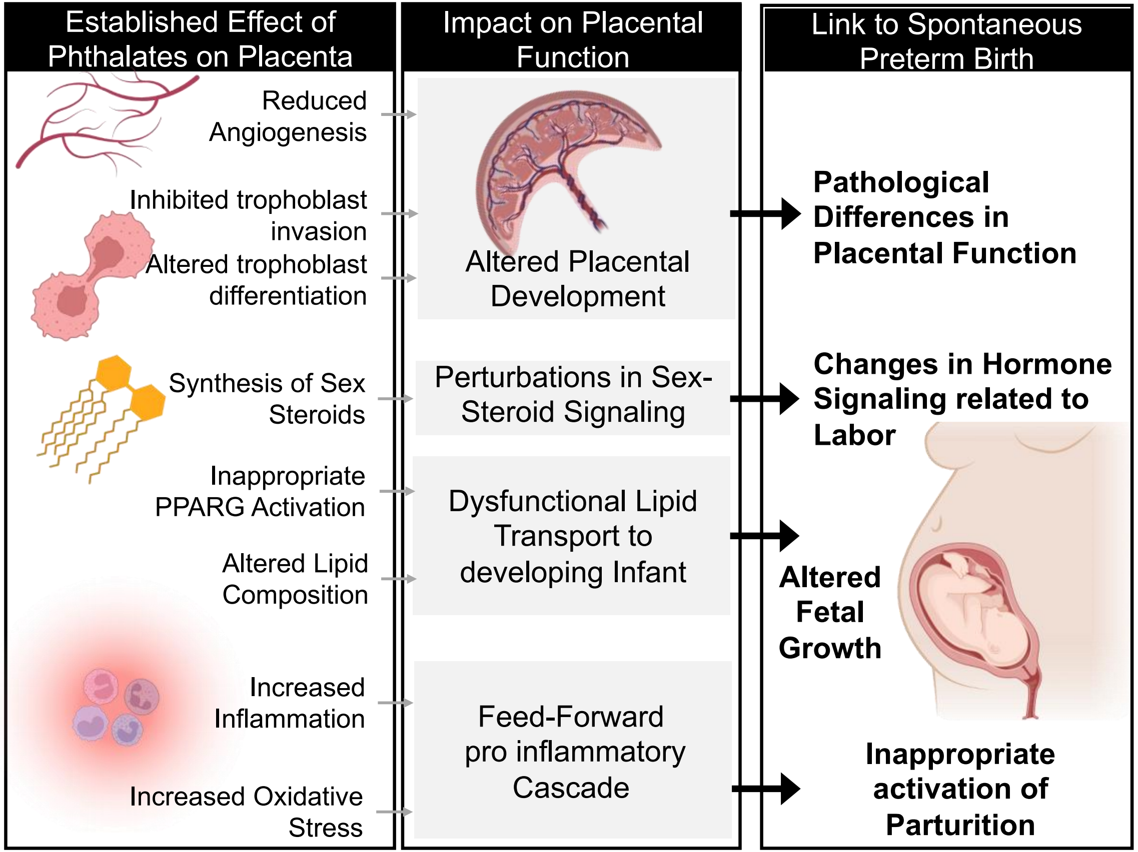
Barrett M. Welch, PhD[1]; Alexander P. Keil, PhD[2]; Jessie P. Buckley, PhD[3]; et al

» Author Affiliations | Article Information

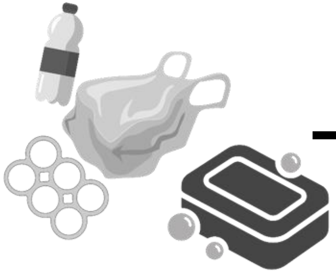
JAMA Pediatr. Published online July 11, 2022.
doi:10.1001/jamapediatrics.2022.2252

- 6045 pregnant women across 16 US studies
- Higher concentrations of most phthalate metabolites were associated with slightly higher odds of preterm birth
- Reducing mixture of phthalate metabolite levels by **50%** could prevent preterm births by **12%** on average

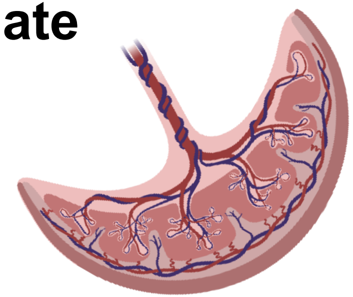
Potential Mechanisms



Hypothesis



Prenatal Phthalate Exposure →



→ **Spontaneous Preterm Birth**

The placenta is the molecular mediator between prenatal phthalate exposure and spontaneous preterm birth

OVERVIEW OF ECHO PATHWAYS

ECHO PATHWAYS: A multi-site, multi-PI consortium

Sheela Sathyanarayana, MD, MPH
Transcriptomics Lead PI

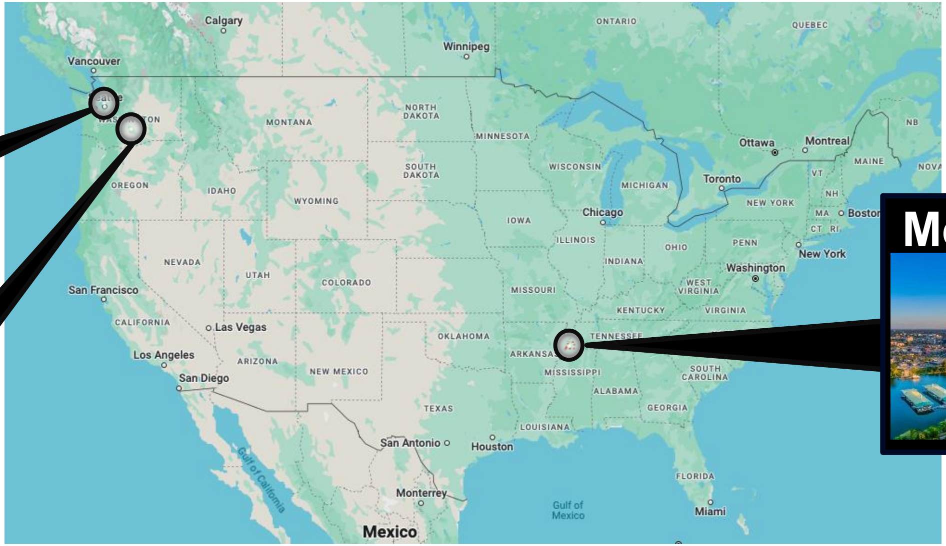
gapps
GLOBAL ALLIANCE TO PREVENT PREMATURITY AND STILLBIRTH

Seattle WA

West Coast

Yakima WA

Rural



THE CANDLE STUDY
UTHSC

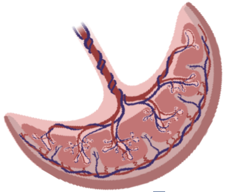
Memphis TN

Urban South

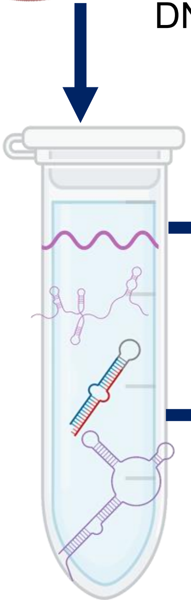
Generating multi-omic data with the UW EDGE center

W INTERDISCIPLINARY CENTER FOR EXPOSURES, DISEASES, GENOMICS AND ENVIRONMENT

Theo Bammler, PhD
Manager: Genomics, Bioinformatics & Biostatistics, Microphysiological Systems Facility Core



Qiagen: AllPrep DNA/RNA Micro Kit



ECHO PATHWAYS (UH/UG3OD023271)
mRNA (N=784 CANDLE; 465 GAPPS)
Sequenced 2016-2023

NIEHS ONES (R01ES033785)
microRNA (N=769 CANDLE)
Sequenced 2022-2024

Transcriptomic Signatures of Prenatal Phthalate Exposure

Vol. 129, No. 9 | **Research**

A Comprehensive Assessment of Associations between Prenatal Phthalate Exposure and the Placental Transcriptomic Landscape

Alison G. Paquette, James MacDonald, Samantha Lapehn, Theo Bammler, Laken Kruger, Drew B. Day, Nathan D. Price, Christine Loftus, Kurunthachalam Kannan, Carmen Marsit, W. Alex Mason, Nicole R. Bush, Kaja Z. LeWinn, Daniel A. Enquobahrie, ... See all authors

Published: 3 September 2021 | CID: 097003 | https://doi.org/10.1289/EHP8973

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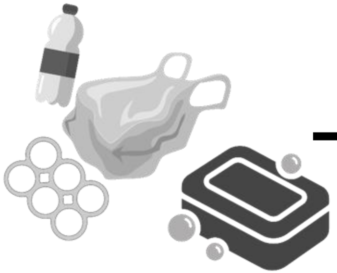
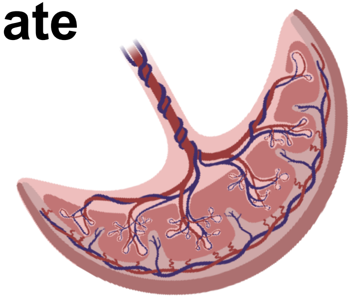
Placental transcriptomic signatures of spontaneous preterm birth

Alison G. Paquette, PhD • James MacDonald, MS • Theo Bammler, PhD • ... Michael Gravett, MD • Daniel A. Enquobahrie, MD, PhD • Sheela Sathyanarayana, MD, MPH • Show all authors

Open Access • Published: July 19, 2022 • DOI: https://doi.org/10.1016/j.ajog.2022.07.015 •

Prenatal Phthalate Exposure

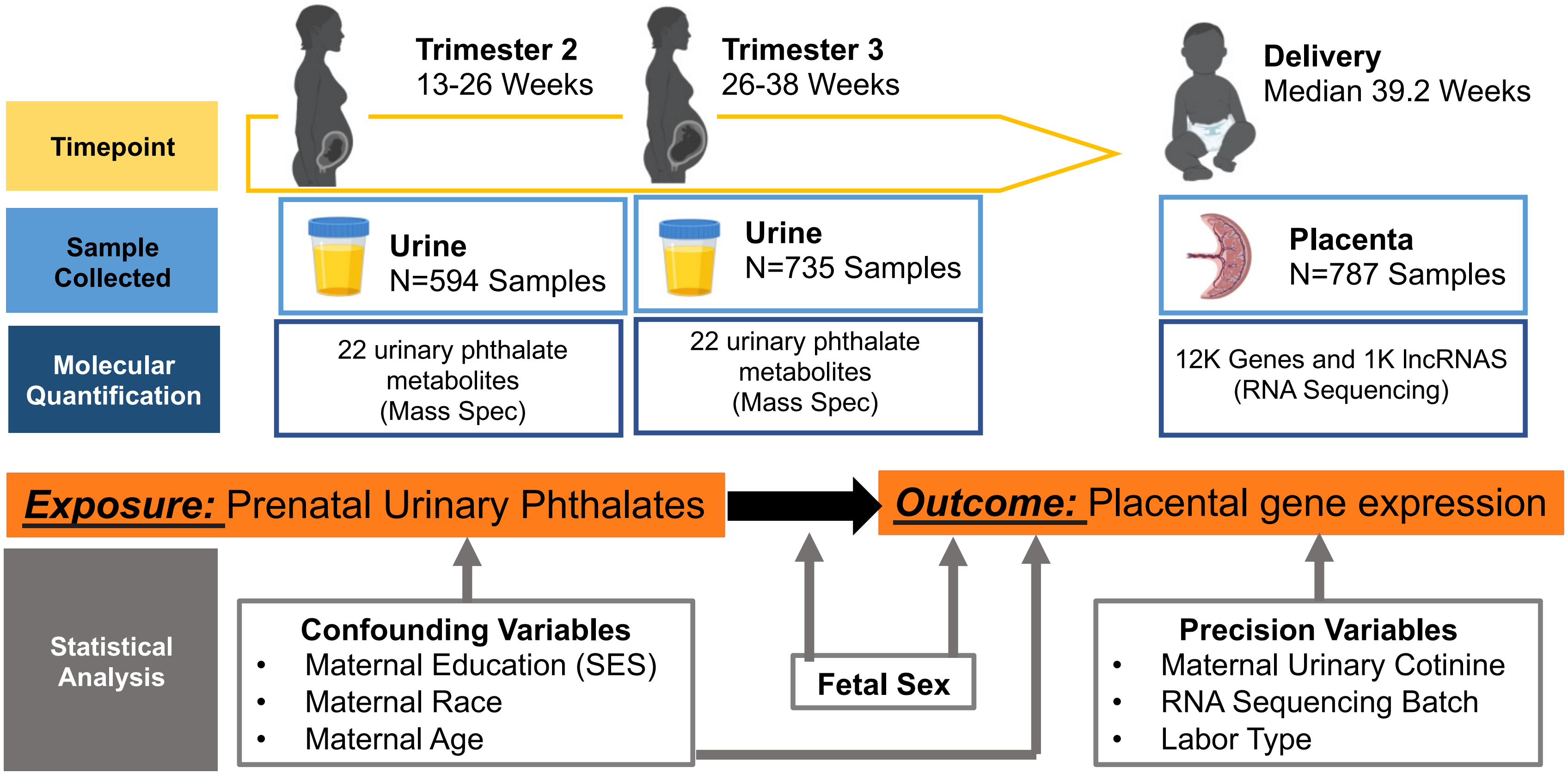




Spontaneous Preterm Birth

The placenta is the molecular mediator between prenatal phthalate exposure and spontaneous preterm birth

Analysis Strategy

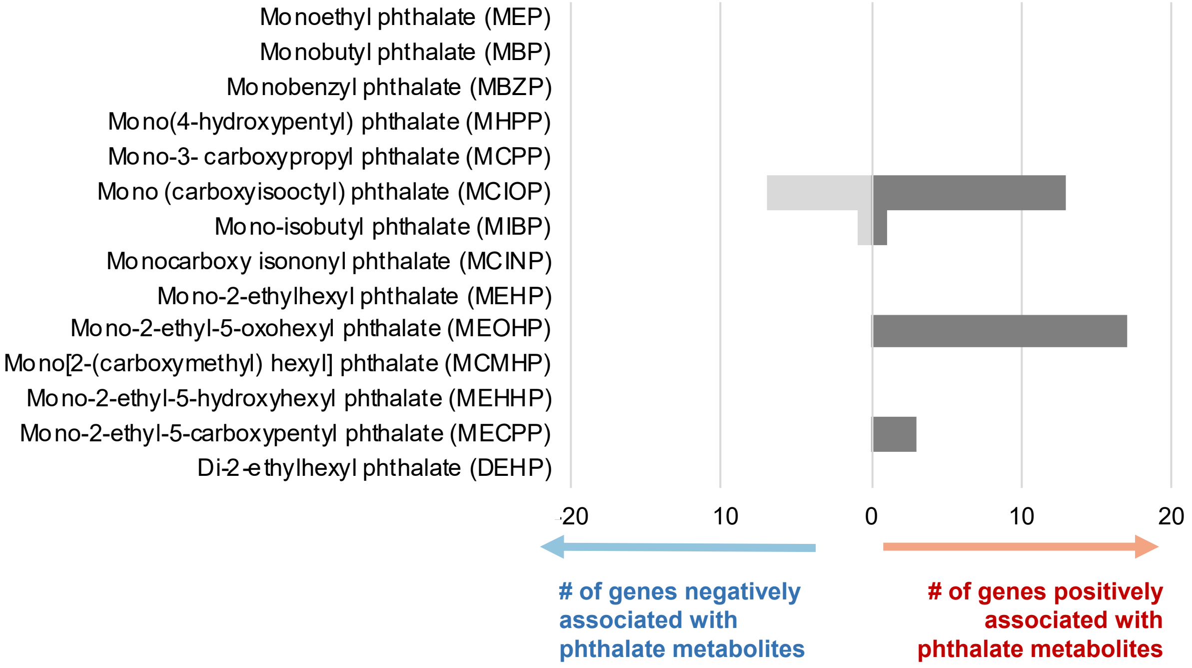


Paquette et al, EHP, 2021

Differentially Expressed Genes (DEGs) and microRNAS (DE miRNAS) associated with Phthalate Metabolites

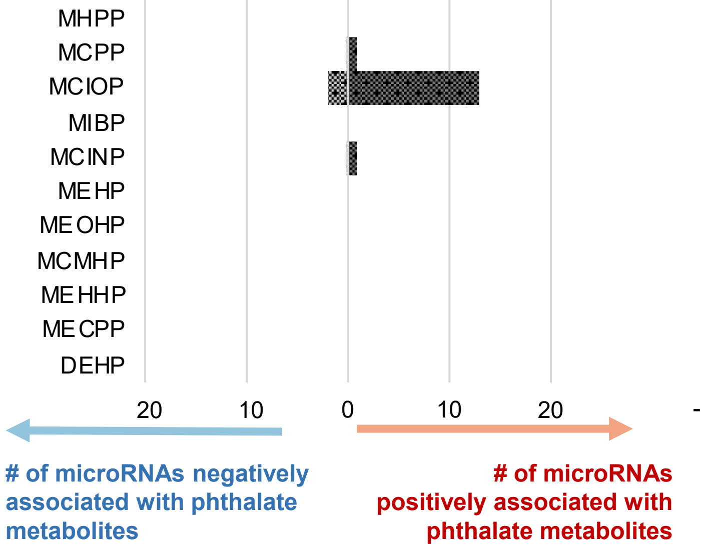
Differentially Expressed Genes *Paquette et al, EHP, 2021*

FDR<0.05



DE microRNAs

FDR<0.1



Samantha Lapehn, PhD

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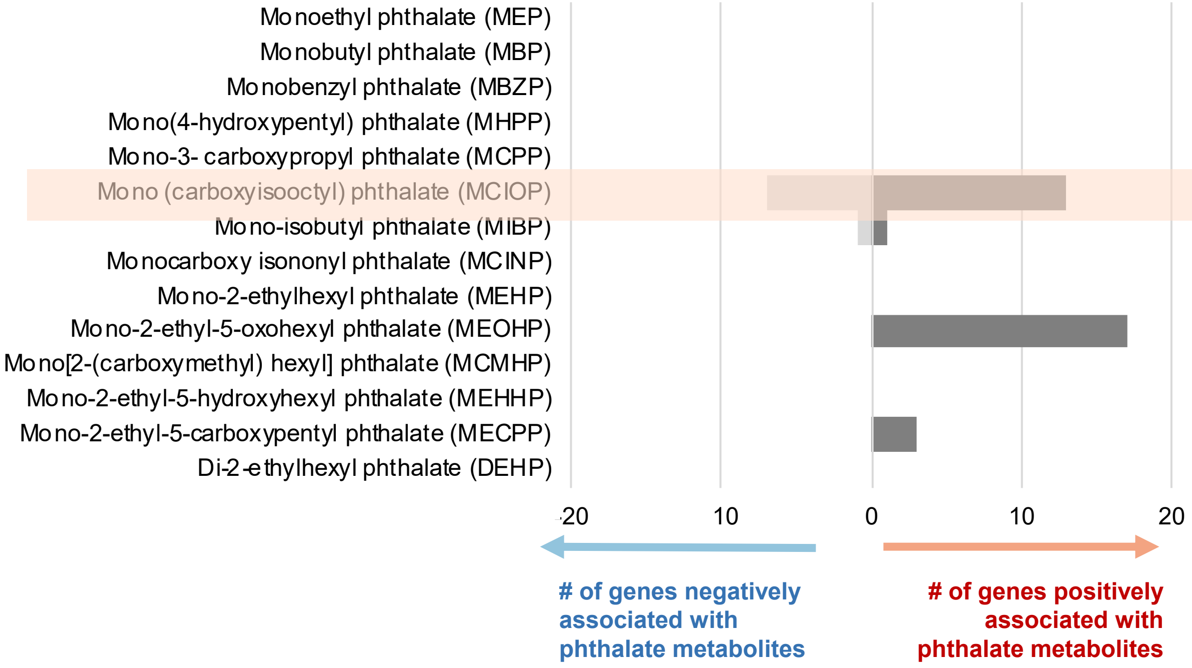
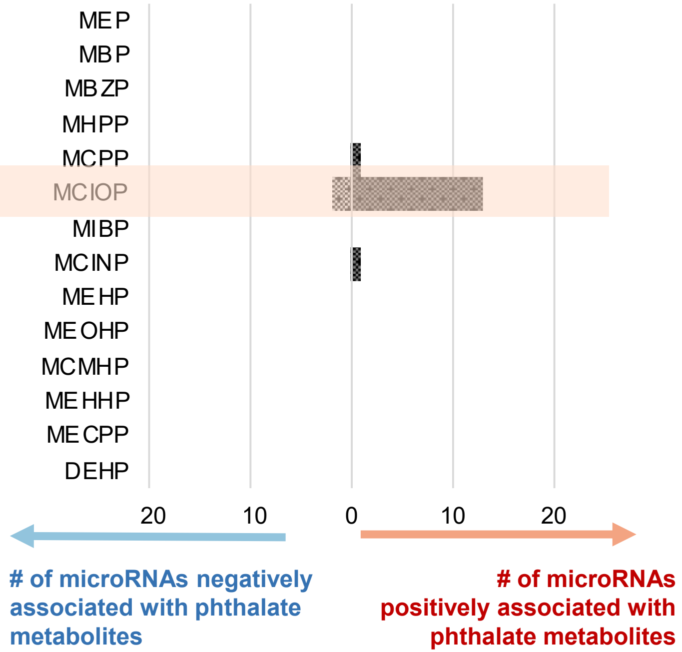


DE microRNAs

FDR<0.1



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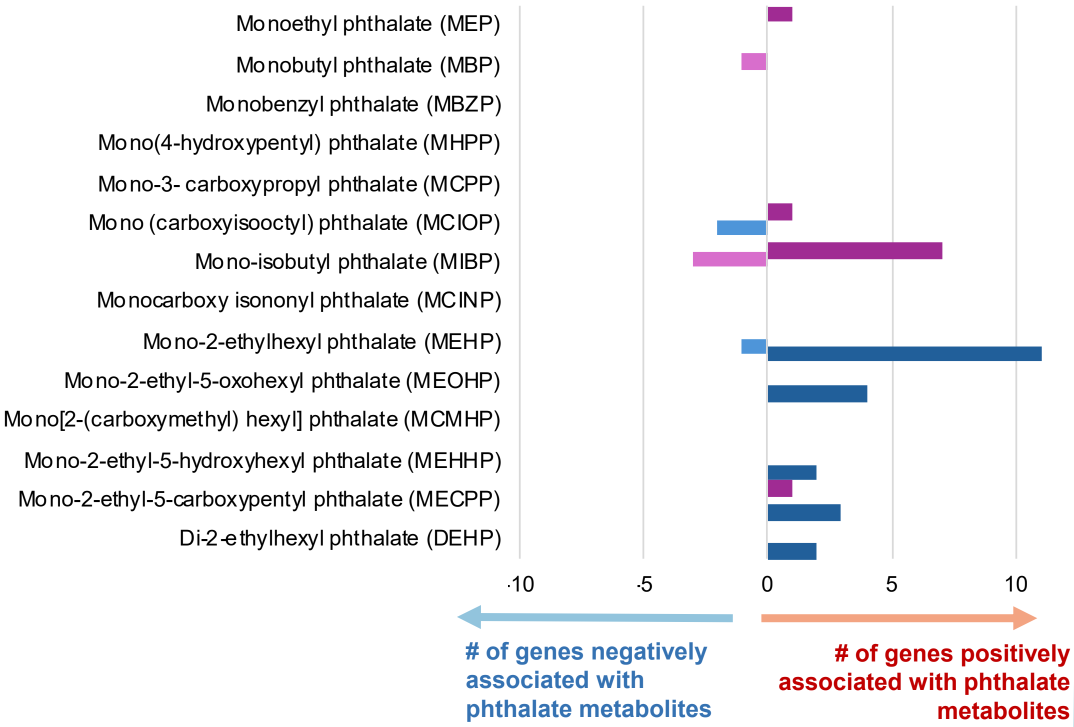
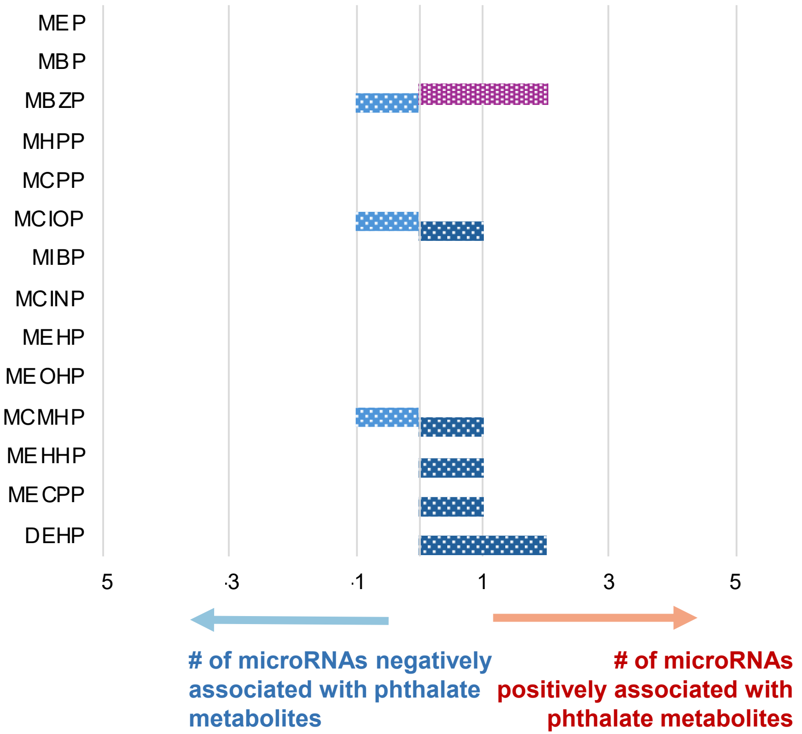


DE microRNAs

FDR<0.1



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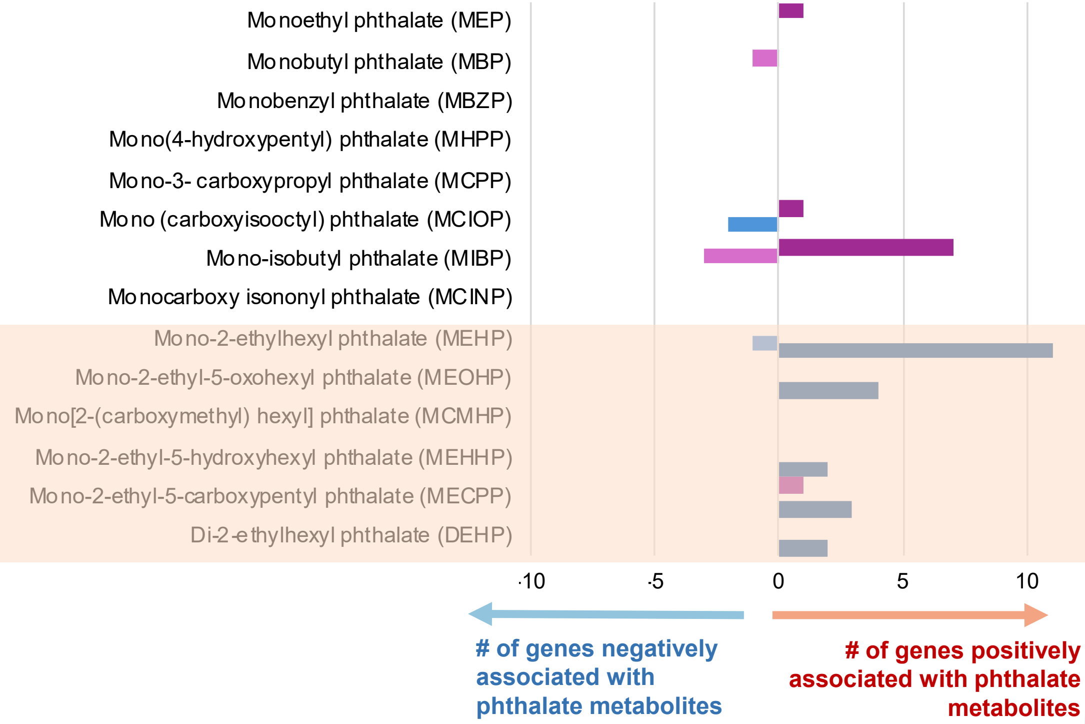
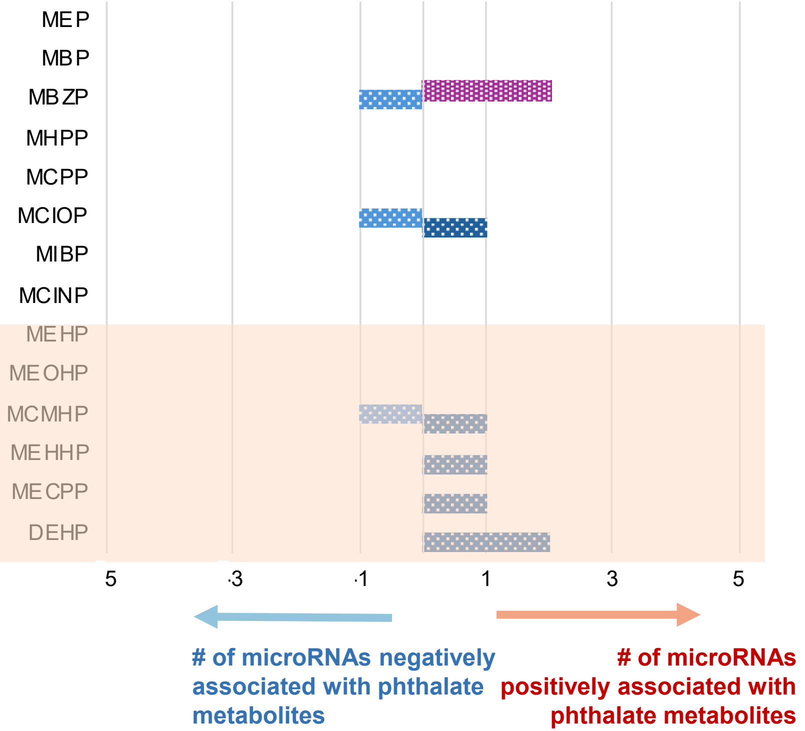
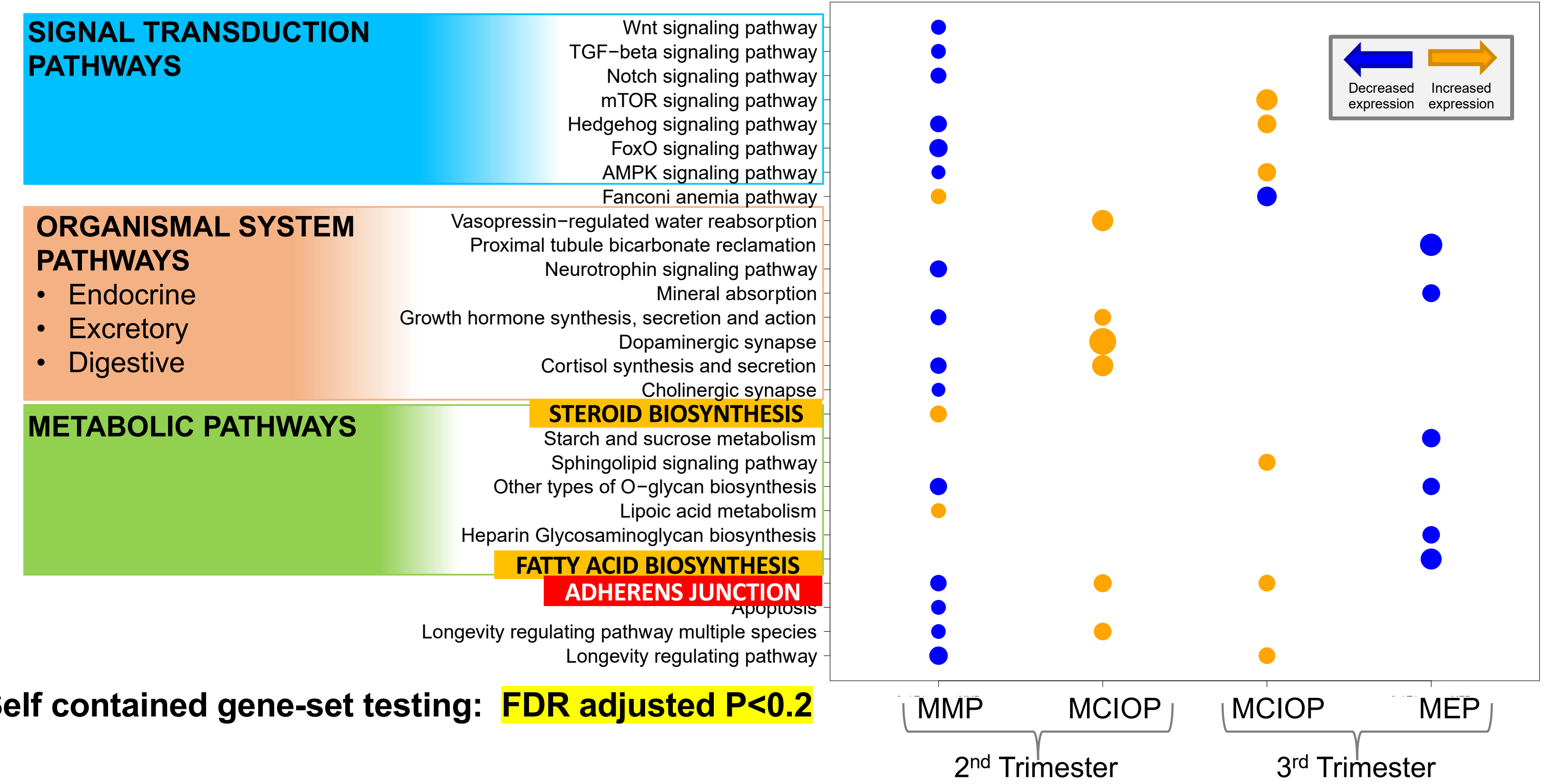


DE microRNAs

FDR<0.1



Biological Pathways Significantly Associated With Phthalate Metabolites



Self contained gene-set testing: **FDR adjusted P<0.2**

Placental Transcriptomic Signatures of spontaneous Preterm Birth


Vol. 129, No. 9 | Research

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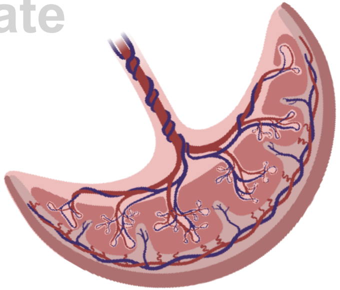
Open Access • Published: July 19, 2022 • DOI: https://doi.org/10.1016/j.ajog.2022.07.015 •

Prenatal Phthalate Exposure



Spontaneous Preterm Birth

The placenta is the molecular mediator between prenatal phthalate exposure and spontaneous preterm birth

Analysis Strategy

Complete Population: CANDLE

Study Design: Prospective cohort study, continuously recruiting (2006-2011)
Location: Memphis TN
Inclusion Criteria:
- Participant age 16-40
- Delivery at participating Hospital

N:1503

Exclusion Criteria
- Multi-fetal Pregnancy
- Complications present at enrollment

Placental Samples Collected: 937

RNA Sequencing: 794

Complete Population: GAPPS

Study Design: Pregnancy Biorepository (2007-present)
Location: Seattle and Yakima WA
Inclusion Criteria:
- Participant age of >18 or Medically emancipated
- Confirmed Pregnancy

N:433

Exclusion Criteria
- Inability to Consent
- >37 weeks or Active labor at time of recruitment

Placental Samples Collected: 295

RNA Sequencing: 289

Study Population:

Study Exclusion Criteria:
- Multi-Fetal Pregnancy
- Induced Labor
- Placental Abruption
- Preeclampsia
- Chorioamnionitis
- Missing Data

CANDLE Participants: 461 (34 Preterm / 427 Term)
GAPPS Participants: 127 (14 Preterm / 113 Term)
Total Study Participants: 588 (48 Preterm / 540 Term)

INDEPENDENT VARIABLE:
sPTB

DEPENDENT VARIABLE:
A. **Transcriptome**[1]
~13K Genes
B. **microRNAs**
557 microRNAs

CONFOUNDING VARIABLES
- Maternal Race
- Maternal Ethnicity
- Maternal Education
- Maternal Smoking status
- Maternal Alcohol consumption
- Maternal Income
- Maternal BMI
- Maternal Age
- Delivery Method
- PPROM

PRECISION VARIABLES
- Fetal Sex
- Sequencing Batch
- Cohort
- Study Site

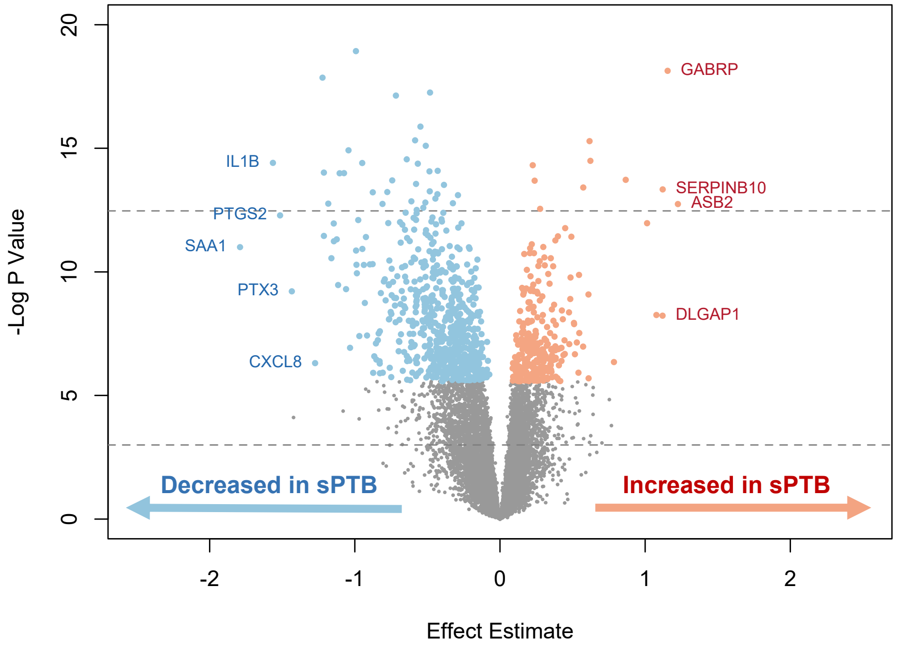
1. Paquette et al, American Journal of Obstetrics and Gynecology 2022

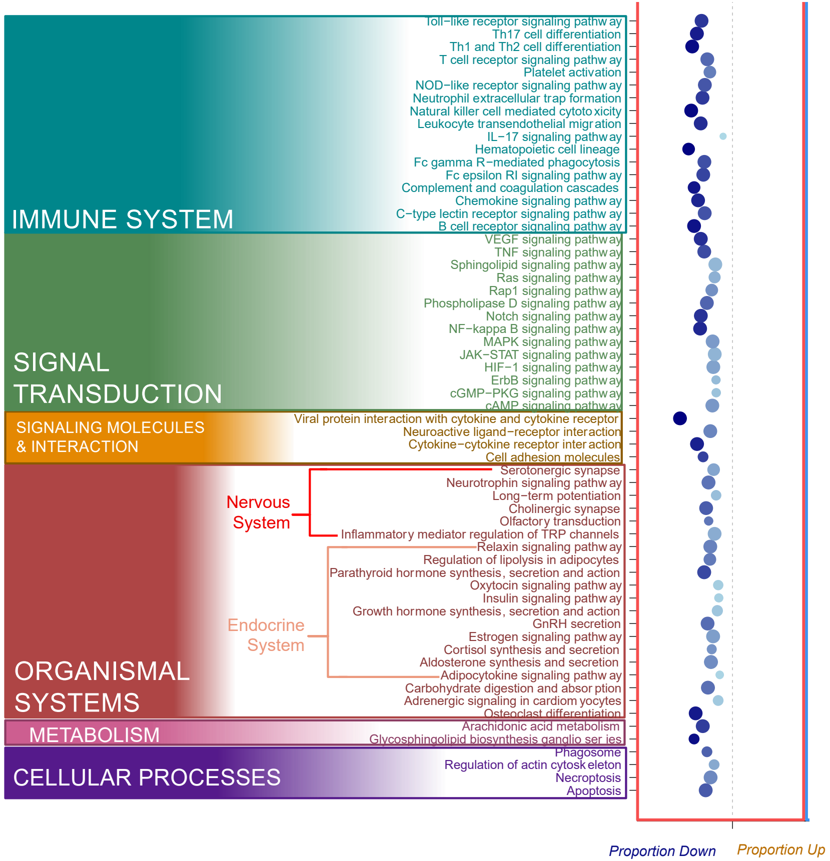
Differentially Expressed Genes (DEGs) and Pathways associated with sPTB

961 Differentially Expressed Genes

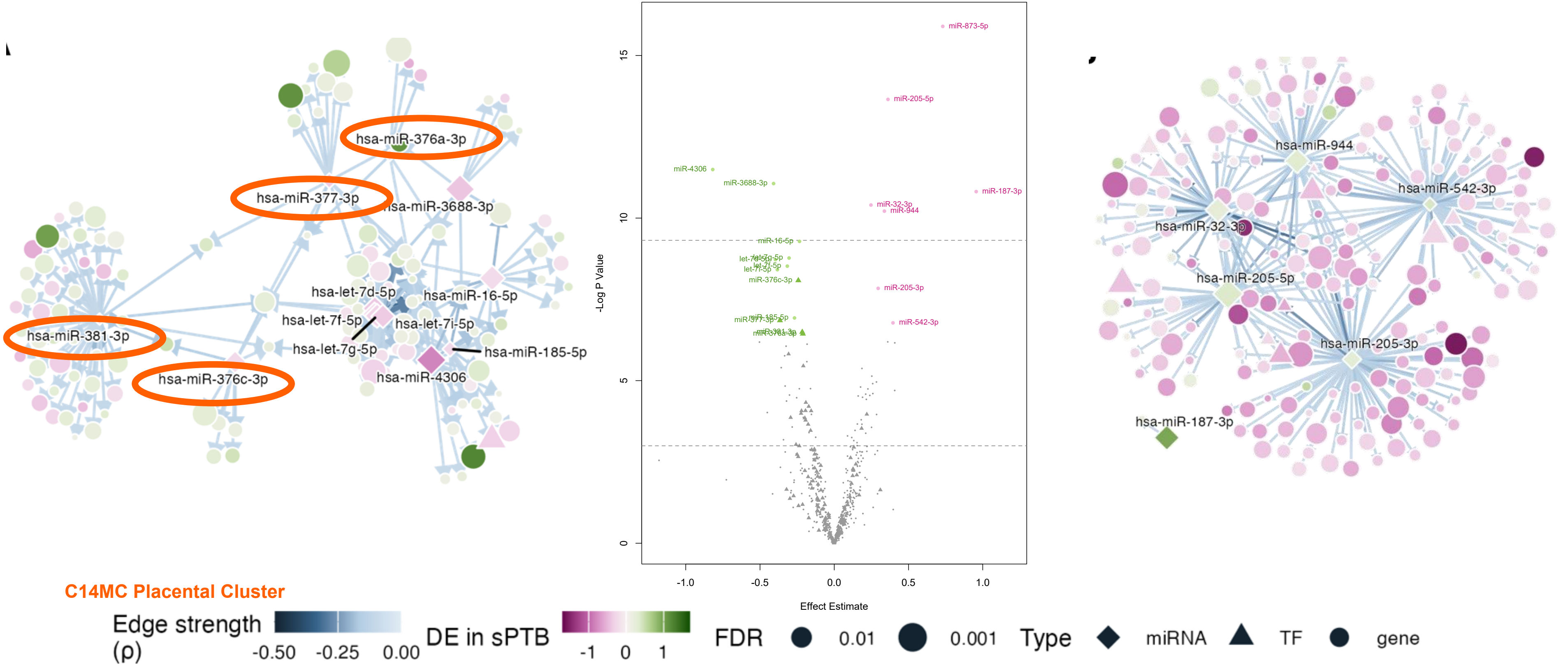
Paquette et al, AJOG, 2023

FDR<0.05



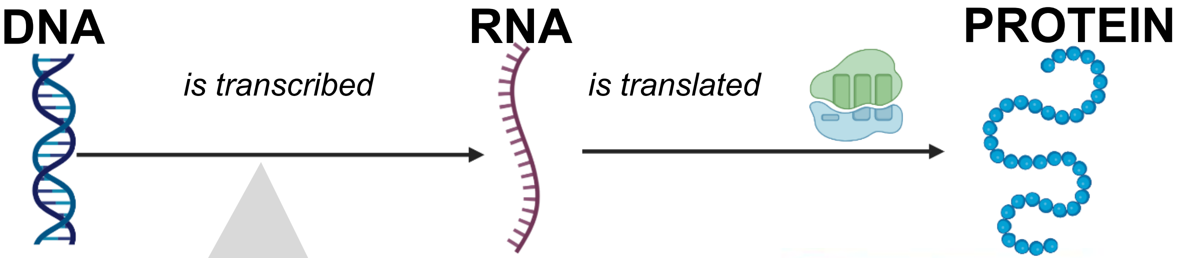


19 Differentially Expressed microRNAs associated with sPTB

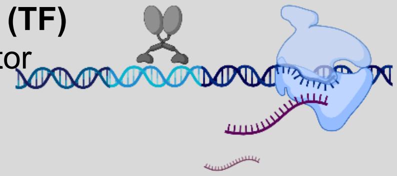


Pre and Post-transcriptional Regulation of Gene Expression

CENTRAL DOGMA



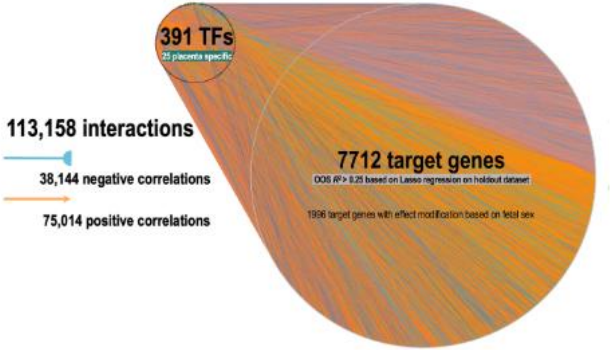
Transcription Factor (TF)
Transcriptional regulator



1. TF and target gene are expressed
2. Binding site for TF in promoter region of gene
   - HOCOMOCO, JASPAR

3. TF and target gene are correlated
   - ***Negatively correlated-*** repressor
   - ***Positively correlated*** -activator

4. TF predicts gene expression in an independent dataset



SCIENCE ADVANCES | RESEARCH ARTICLE

DEVELOPMENTAL BIOLOGY

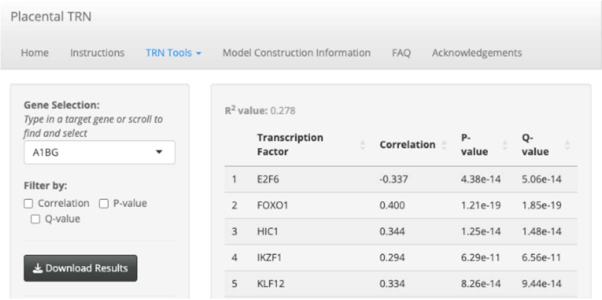
A genome scale transcriptional regulatory model of the human placenta

Alison Paquette[1,2]*, Kylia Ahuna[3], Yeon Mi Hwang[4], Jocelynn Pearl[5], Hanna Liao[1], Paul Shannon[4], Leena Kadam[3], Samantha Lapehn[2], Matthew Bucher[3], Ryan Roper[4], Cory Funk[4], James MacDonald[1], Theo Bammler[1], Priyanka Baloni[4]†, Heather Brockway[6]‡, W. Alex Mason[7], Nicole Bush[8], Kaja Z. Lewinn[8], Catherine J. Karr[1], John Stamatoyannopoulos[1,9], Louis J. Muglia[10,11], Helen Jones[12], Yoel Sadovsky[13,14], Leslie Myatt[3], Sheela Sathyanarayana[1,2], Nathan D. Price[4,15]

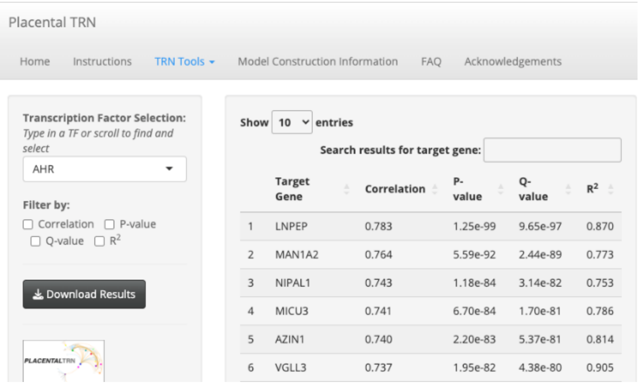
ECHO OIF: 0035
2019-2021

TRN Web Tool: A resource for ECHO Investigators

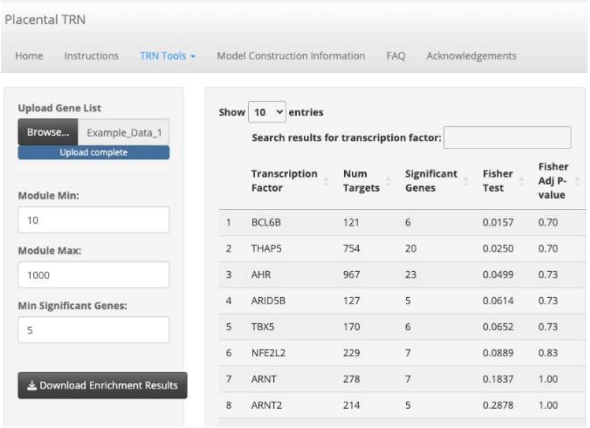
1. Identify the TFs of a given target gene

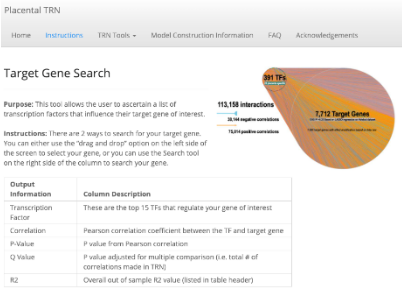


2. Identify the target genes of a given TF



3. Perform TF Enrichment Analysis for a list of target genes





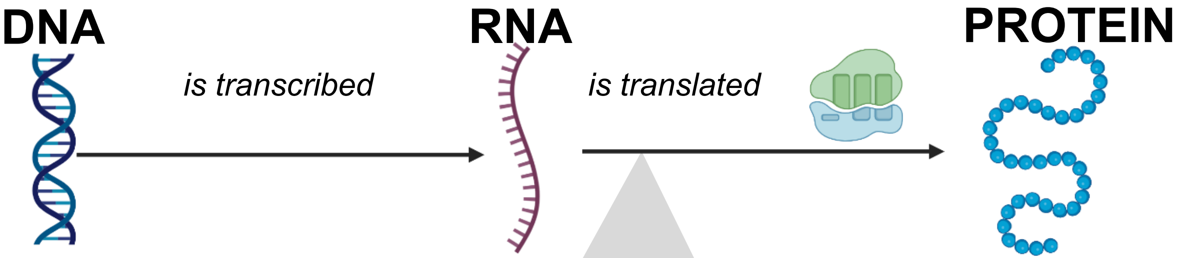
-instructions
-model construction information
-frequently asked questions

Evan Firsick, MA

https://paquettelab.shinyapps.io/TRNAp

Pre and Post-transcriptional Regulation of Gene Expression

CENTRAL DOGMA

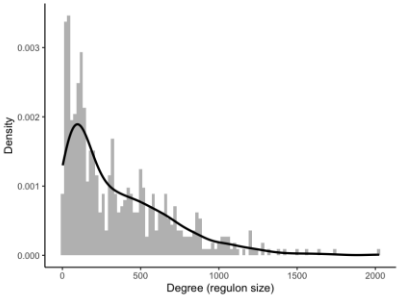


microRNA
Post-transcriptional regulator

1. microRNA and target gene are expressed
2. microRNA binds to target gene
   - Targetscan, mirDB
3. TF and target gene are ***negatively*** correlated

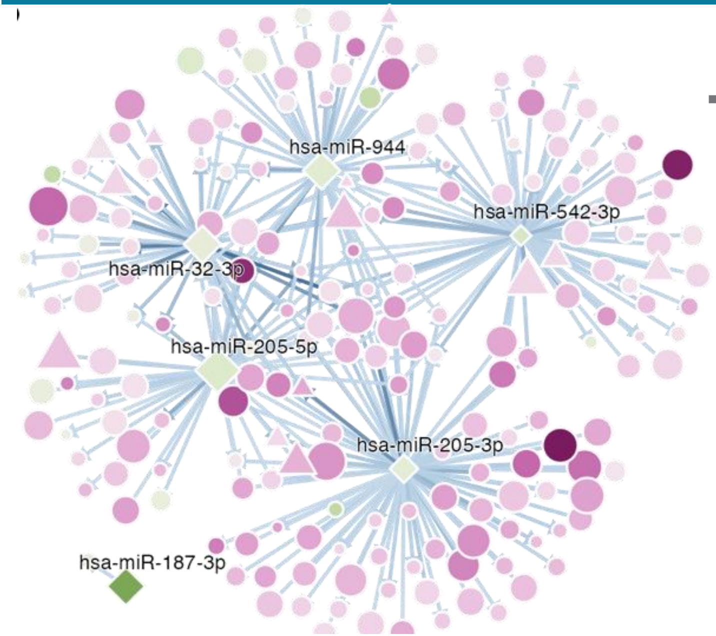
Placental microRNA Regulatory Network

- 563 miRNA
- 11,771 target genes
- 199,188 miRNA–mRNA interactions (FDR<0.05)
- *Shiny app: Coming soon!*



Mariana Parenti, PhD

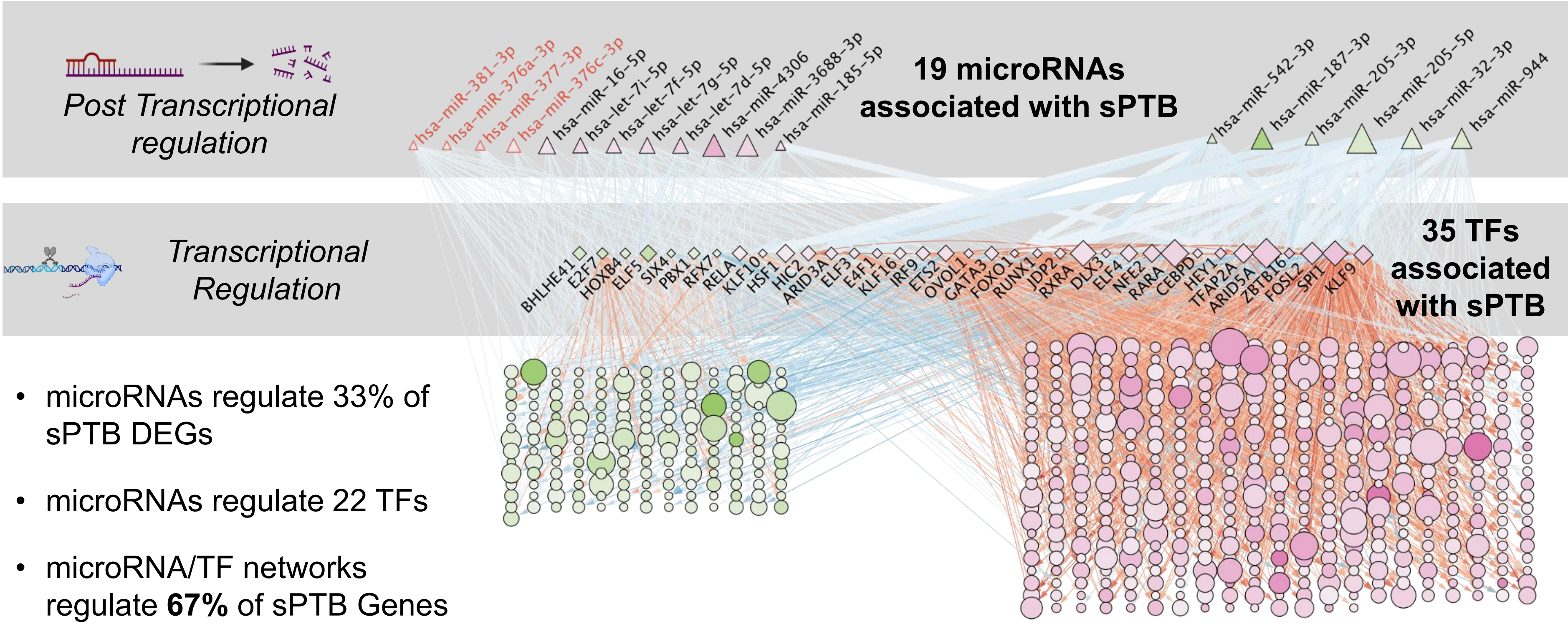
Placental microRNAs provide insight into Perturbed Pathways



Gene set over-representation analysis (FDR<0.1)

Pathways	FDR	sPTB Genes /Target Genes
Th17 cell differentiation	0.0334	8/74
B cell receptor signaling pathway	0.0555	7/67
Fc epsilon RI signaling pathway	0.0851	7/85
Hematopoietic cell lineage	0.0851	5/47
Natural killer cell mediated cytotoxicity	0.0851	6/73
Intestinal immune network for IgA production	0.0851	4/25
Fc gamma R-mediated phagocytosis	0.0851	5/51
Antigen processing and presentation	0.0851	5/46
TNF signaling pathway	0.0825	8/99
VEGF signaling pathway	0.0851	5/48
cAMP signaling pathway	0.0851	8/125
Neuroactive ligand-receptor interaction	0.0851	7/99
Osteoclast differentiation	0.0851	8/111

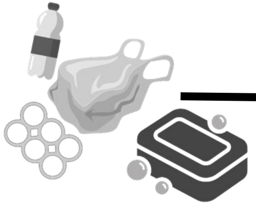
Network Biology Approaches Yield Additional Insight into the Etiology of sPTB

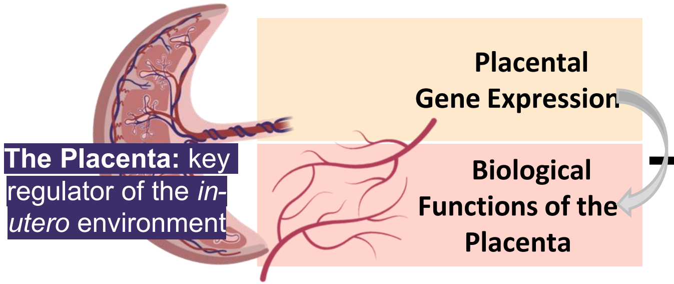


- microRNAs regulate 33% of sPTB DEGs
- microRNAs regulate 22 TFs
- microRNA/TF networks regulate **67%** of sPTB Genes (625/961)

Integrating Signatures to Identify Shared Mechanisms

Prenatal Phthalate Exposure

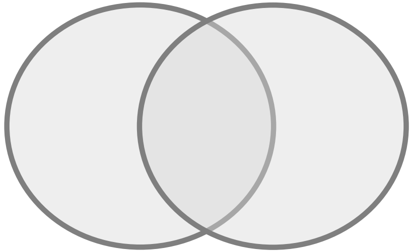




Spontaneous Preterm Birth

Meet in the Middle Strategy:

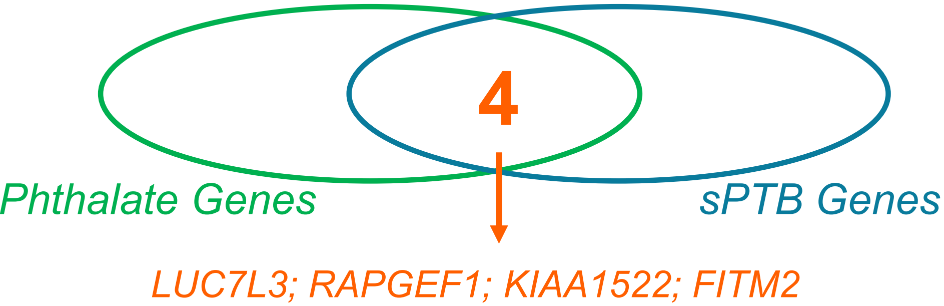
Genes and Pathways associated with phthalates



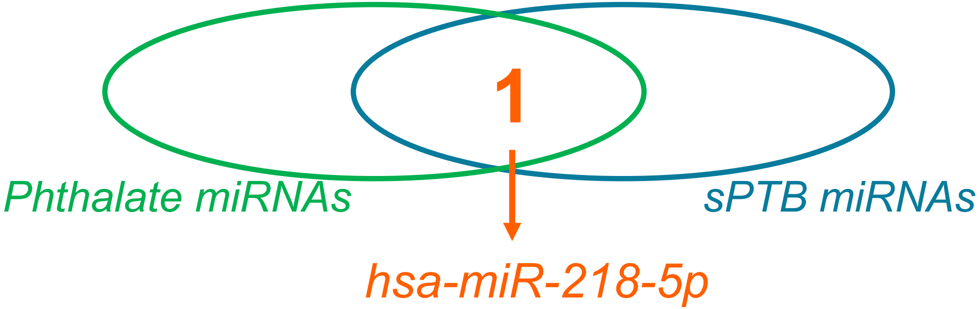
Genes and Pathways associated with sPTB

Meet in the Middle Results

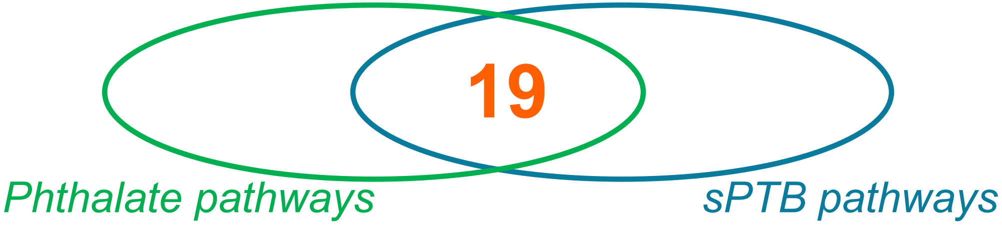
1. Genes



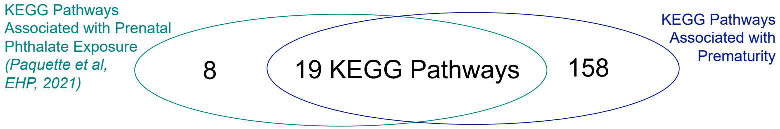
2. microRNAs



3. Pathways



Prenatal Phthalate Exposure and sPTB: Overlapping Pathway Signatures



		Phthalate Analysis				sPTB Analysis		
		Metabolite	Trimester Collected	FDR Adjusted P	Direction	Preterm Birth Group	FDR Adjusted P	Direction
METABOLISM	Sphingolipid signaling pathway	MCIOP MCIOP	Trimester 2 Trimester 3	0.125 0.177	Up Up	Preterm vs. Term Early Preterm vs. Full Late Preterm vs. Full	Down Down Down	3.01E-05 1.35E-03 1.82E-03
	Steroid biosynthesis	MMP	Trimester 2	0.160	Up	Preterm vs. Term Early Preterm vs. Full	Up Up	6.51E-04 1.44E-04
	Other types of O-glycan biosynthesis	MEP	Trimester 3	0.160	Down	Preterm vs. Term Early Preterm vs. Full	Down Down	2.52E-03 1.44E-04
SIGNAL TRANSDUCTION	Notch signaling pathway	MMP	Trimester 2	0.160	Down	Preterm vs. Term Early Preterm vs. Full Late Preterm vs. Full	Down Down Down	3.01E-05 5.06E-05 4.14E-03
	AMPK signaling pathway	MCIOP	Trimester 3	0.177	Up	Preterm vs. Term Late Preterm vs. Full	Down Down	5.48E-03 4.31E-02
	FoxO signaling pathway	MMP	Trimester 2	0.160	Down	Preterm vs. Term Early Preterm vs. Full	Down Down	3.77E-03 2.19E-04
	Wnt signaling pathway	MMP	Trimester 2	0.160	Down	Preterm vs. Term Early Preterm vs. Full	Down Down	7.29E-03 1.12E-04
	mTOR signaling pathway	MCIOP	Trimester 3	0.177	Up	Preterm vs. Term Early Preterm vs. Full	Down Down	2.46E-02 4.43E-04
	TGF-beta signaling pathway	MMP	Trimester 2	0.160	Down	Preterm vs. Term Early Preterm vs. Full	Down Down	3.02E-02 1.48E-03
CELLULAR PROCESSES	Fanconi anemia pathway	MCIOP MMP	Trimester 3 Trimester 2	0.177 0.160	Down Up	Preterm vs. Term	Up	4.81E-03
	Adherens junction	MCIOP MCIOP	Trimester 3 Trimester 3	0.177 0.125	Up Up	Preterm vs. Term Early Preterm vs. Full	Down Down	2.64E-02 1.52E-02
	Longevity regulating pathway	MCIOP MCIOP	Trimester 2 Trimester 3	0.160 0.177	Down Up	Preterm vs. Term Early Preterm vs. Full	Down Down	2.99E-04 4.43E-04
	Vasopressin-regulated water reabsorption	MCIOP	Trimester 2	0.096	Up	Preterm vs. Term Late Preterm vs. Full	Down Down	4.04E-02 3.27E-02
	Dopaminergic synapse	MCIOP	Trimester 2	0.047	Up	Preterm vs. Term Late Preterm vs. Full	Down Down	2.61E-02 3.40E-02
ENDOCRINE SYSTEMS	Growth hormone synthesis, secretion and action	MCIOP	Trimester 2	0.125	Up	Preterm vs. Term Early Preterm vs. Full Late Preterm vs. Full	Down Down Down	2.23E-04 1.12E-04 8.68E-03
	Cortisol synthesis and secretion	MMP	Trimester 2	0.160	Down	Preterm vs. Term Early Preterm vs. Full Late Preterm vs. Full	Down Down Down	5.48E-04 1.90E-03 8.89E-03
	Neurotrophin signaling pathway	MMP	Trimester 2	0.160	Down	Preterm vs. Term Early Preterm vs. Full Late Preterm vs. Full	Down Down Down	3.01E-05 2.99E-04 1.72E-03
	Cholinergic synapse	MMP	Trimester 2	0.183	Down	Preterm vs. Term Early Preterm vs. Full Late Preterm vs. Full	Down Down Down	3.01E-05 5.06E-05 2.69E-03

Hormones Involved in Parturition

↑ **Steroid Biosynthesis**

↓ **Cortisol synthesis & secretion**

Signaling for Placental Growth & development

↓ **TGF Beta Signaling**

↓ **Notch Signaling**

Oxidative Stress

↓ **FOXO1 Signaling**

Key Take Aways

1. Developmental Origins of Health and Disease (***DOHAD***): The prenatal environment is regulated by the ***placenta*** and shapes infant & childhood health outcomes

2. Prenatal ***phthalate exposure*** is associated with placental dysfunction and ***sPTB*** in epidemiological studies

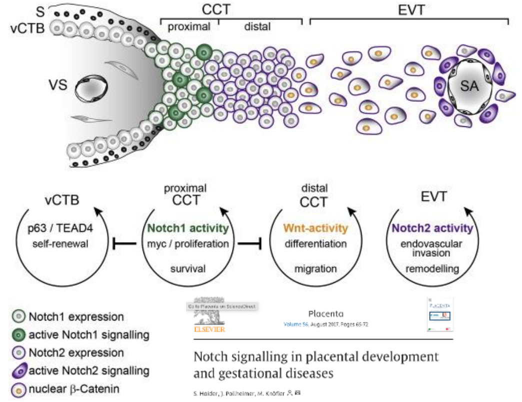
3. We have identified changes in placental gene and microRNA expression attributable to phthalate exposure

4. We have identified a substantial number of transcriptomic differences related to ***sPTB.*** We have used network tools to map these genes to selected TFs and microRNAs

5. We identified **19 pathways** with shared transcriptional disruption related to prenatal phthalate exposure & sPTB, including **metabolic**, **immune signaling**, and **endocrine** pathways

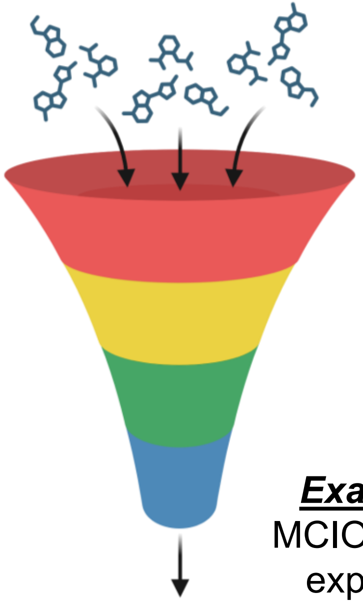
How can Placental Omics data inform regulatory decisions and promote precision environmental health?

Novel insight into molecular mechanisms underlying toxicity



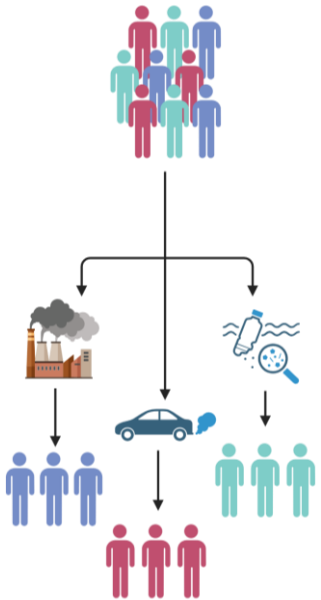
Example: Notch Signaling and Placental Function

Chemical screening to evaluate effects on pregnancy



Example:
MCIOP/DINP exposure

Informing Precision Environmental Health approaches



Example:
Genetic Variability in genes/ microRNAs may exacerbate effects

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