Integrative Exposomic, Transcriptomic, Epigenomic Analyses of Human Placental Tissues Links Understudied Chemicals to Preeclampsia
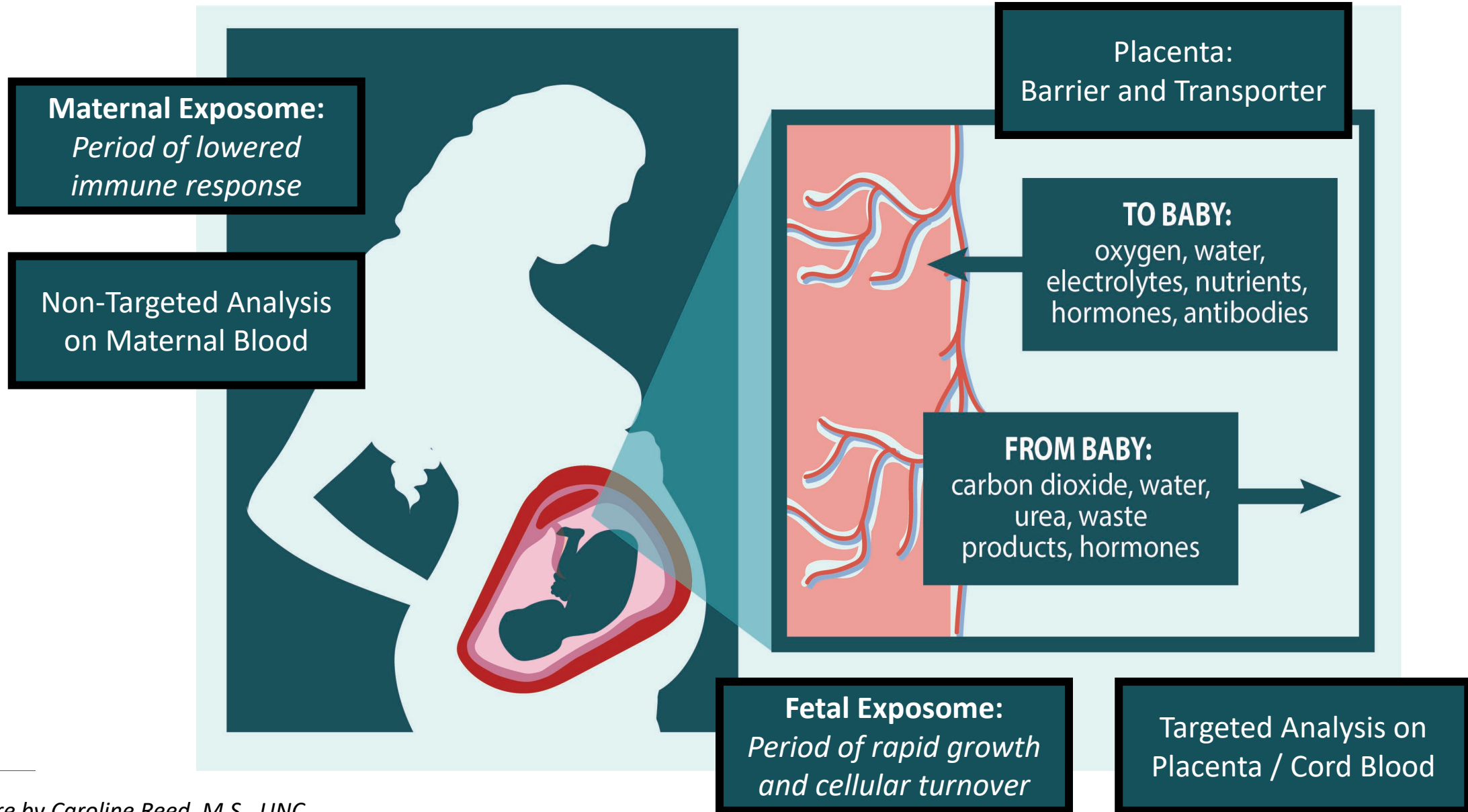
Alex Chao

Jarod N. Grossman, Celeste Carberry, Yunjia Lai, Antony J. Williams, Jeffrey M. Minucci, S. Thomas Purucker, John T. Szilagyi, Kun Lu, Kim Boggess, Rebecca C. Fry, Jon R. Sobus, Julia E. Rager
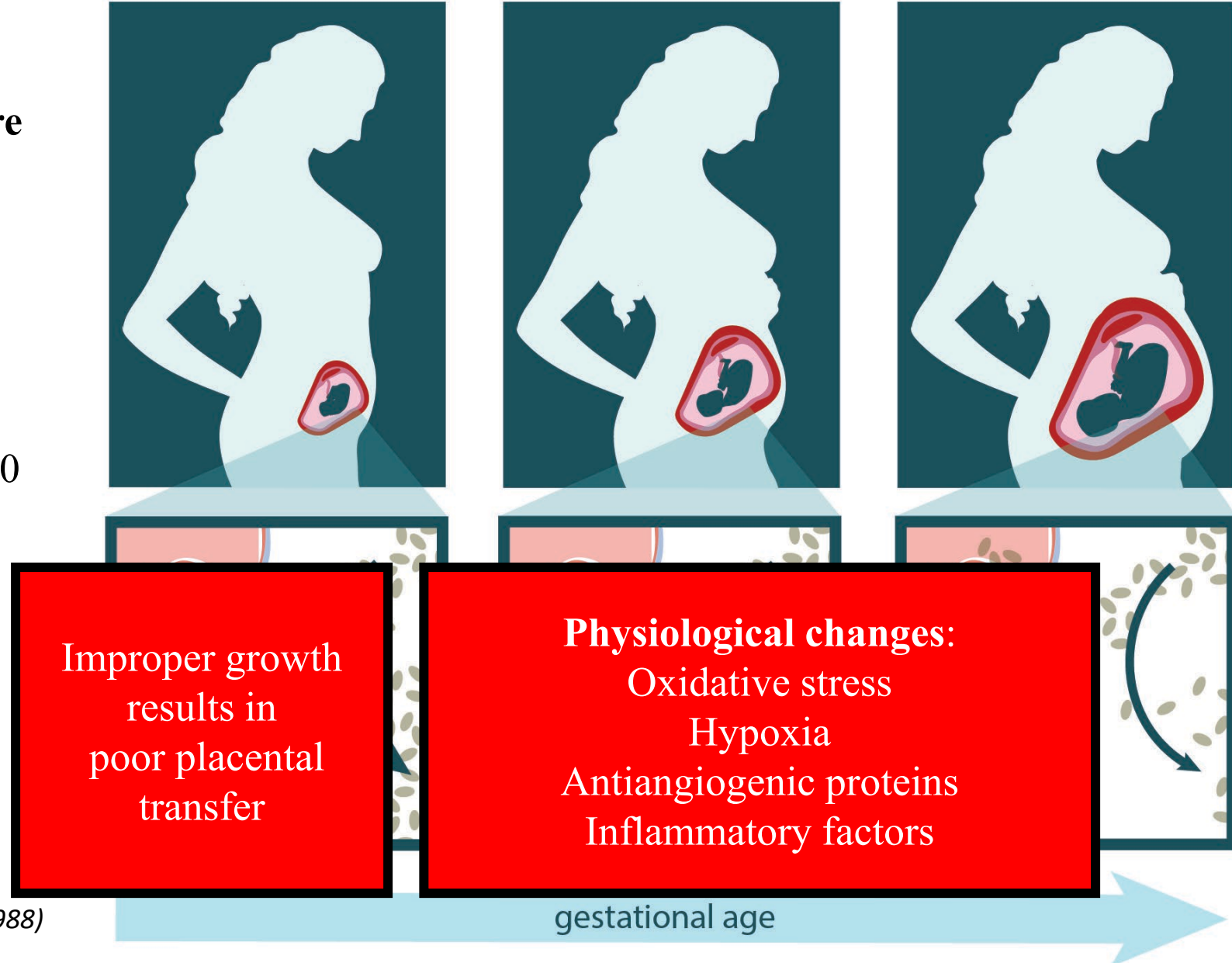
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Pregnancy: Two Susceptible Populations

Maternal Exposome:
Period of lowered immune response

Non-Targeted Analysis on Maternal Blood

Placenta:
Barrier and Transporter

TO BABY:
oxygen, water, electrolytes, nutrients, hormones, antibodies

FROM BABY:
carbon dioxide, water, urea, waste products, hormones

Fetal Exposome:
Period of rapid growth and cellular turnover

Targeted Analysis on Placenta / Cord Blood

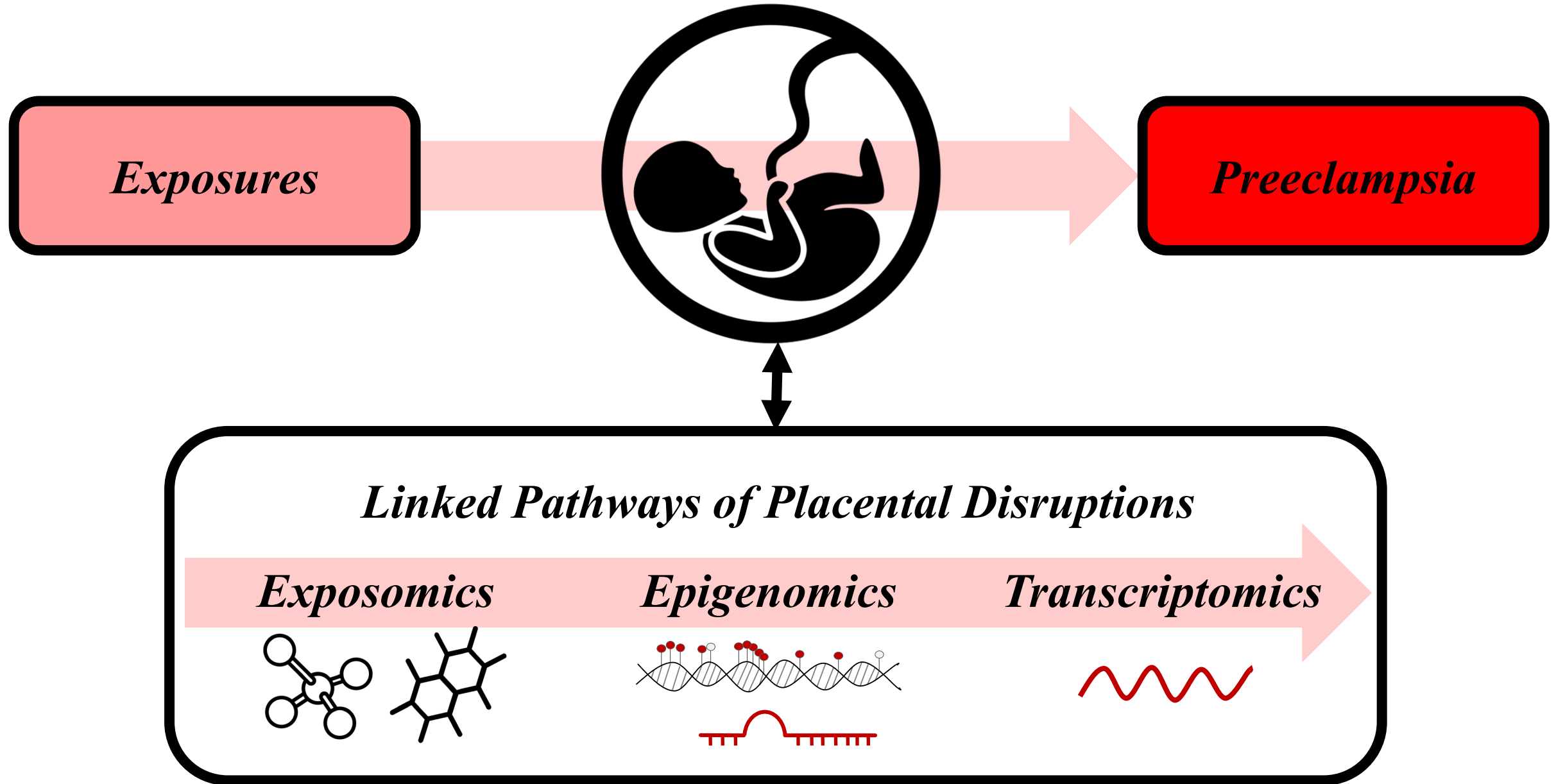*Figure by Caroline Reed, M.S., UNC*

Preeclampsia: A Serious Pregnancy Disorder

o Preeclampsia is a prenatal disease characterized by **high blood pressure** and elevated urine protein levels (indicative of **organ damage**)

o Preeclampsia affects up to 8% of pregnancies worldwide

o Deaths of ~70,000 women / ~500,000 fetuses yearly

o Exact causes are still unknown

o Associated with abnormal formation and growth of the placenta

Improper growth results in poor placental transfer

Physiological changes:
Oxidative stress
Hypoxia
Antiangiogenic proteins
Inflammatory factors

gestational age

(Ananth et al. 2013; Duley 2009; English et al. 2015; WHO 1988)
Figure by Caroline Reed, M.S., UNC

Primary Study Cohort

o Cross-sectional study organized by Dr. Kim Boggess (UNC Maternal and Child Health) and Dr. Rebecca C. Fry (UNC Institute for Environmental Health Solutions)

o Cohort of patients with normotensive (N=17) and preeclamptic (N=18) pregnancies

Characteristics	All N (%) / Mean (range)	Controls N (%) / Mean (range)	Cases N (%) / Mean (range)
Subjects	35 (100%)	17 (100%)	18 (100%)
Race			
African American	13 (37%)	5 (29%)	8 (44%)
Asian	2 (6%)	2 (12%)	0 (0%)
Caucasian	10 (29%)	5 (29%)	5 (28%)
Hispanic	7 (20%)	5 (29%)	2 (11%)
Other	3 (9%)	0 (0%)	3 (17%)
Parity			
Primipara	20 (57%)	9 (53%)	11 (61%)
Multipara	15 (43%)	8 (47%)	7 (39%)
Smoking Status			
Smoker	3 (9%)	1 (6%)	2 (11%)
Non-Smoker	32 (91%)	16 (94%)	16 (89%)
Maternal Age (years)	28.2 (19-38)	27.5 (19-38)	28.6 (19-37)
Gestational age (weeks)	35.3 (22-41)	38.9 (36-41)	31.9 (22-38)

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Non-Targeted Analysis Method

o Mobile Phases: 0.1% Formic Acid in DI water / 0.1% Formic Acid in acetonitrile (w/ 0.5 mM ammonium fluoride in negative mode)

o Zorbax Eclipse Plus C8 1.8 µm 2.1 × 100 mm

o Column temperature: 40°C

o 2.0 µL sample injection

o Samples acquired in triplicate for MS1 data

o Data-dependent acquisition run for MS2 data

o Run in positive and negative mode

Agilent 6546 Q-TOF

Analytical Filtering of Data

Which features observed are real?

Agilent Profinder / Mass Profiler Professional: *Feature generation and retention time alignment*

EPA NTA WebApp: *Feature filtering*

- o Adducts
- o Duplicate features
- o Reproducibility
- o Blank subtraction
- o Sample/blank thresholds

~4000 Features

Statistical Filtering of Data

Which features change in correlation with Preeclampsia observations?

o Normotensive (N=17) and preeclamptic (N=18) pregnancies

o Significance threshold ($\log_{10}$-space) = 1.36

o Fold change threshold ($\log_2$-space) = 1

183 Molecular Features

Epigenomic / Transcriptomic Associations

Correlate epigenomic / transcriptomic levels with molecular feature abundances

730 differentially expressed genes

617 differentially methylated genes

631 differentially expressed miRNAs

Feature and Sample Cluster Results

How can the remaining features and samples be further grouped/prioritized?

Unsupervised Machine Learning Clustering

Preeclampsia "Omics" data

- Samples cluster into three groups of preeclamptic placenta samples, and one containing control samples (plus one preeclamptic)

- Features cluster into eight groups
  - Cluster 7 contains highest association with preeclamptic Omics

Chemical Candidate Prioritization

For each feature, what are the potential chemicals (candidates)?

o Search by accurate mass within EPA's **DSSTox** database

o 183 features → ~6000 candidates

Can we prioritize candidates to investigate?

o MS2 spectral matching
o Exposure database metadata
o Multi-Omics scoring

o **46 chemicals** prioritized and acquired

https://comptox.epa.gov/dashboard

Targeted Confirmation Analysis

o LC conditions matched NTA LC method

o MS1 and MS2 data collected

o Subset of placental samples re-run for retention time and accurate mass alignment

o *23 unique chemicals* confirmed at level 1 identification via matching of accurate mass, retention time and MS2 fragment ions

o Additional matching on *2 features* which consisted of isomeric pairs (*4 unique chemicals*) unable to be chromatographically resolved

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Chemistry and Analytical Core

Thermo Q-Exactive Orbitrap

Chemical ID and Categorization Results

53 total features (corresponding to **40 unique chemicals**) identified at levels 1-3 confidence
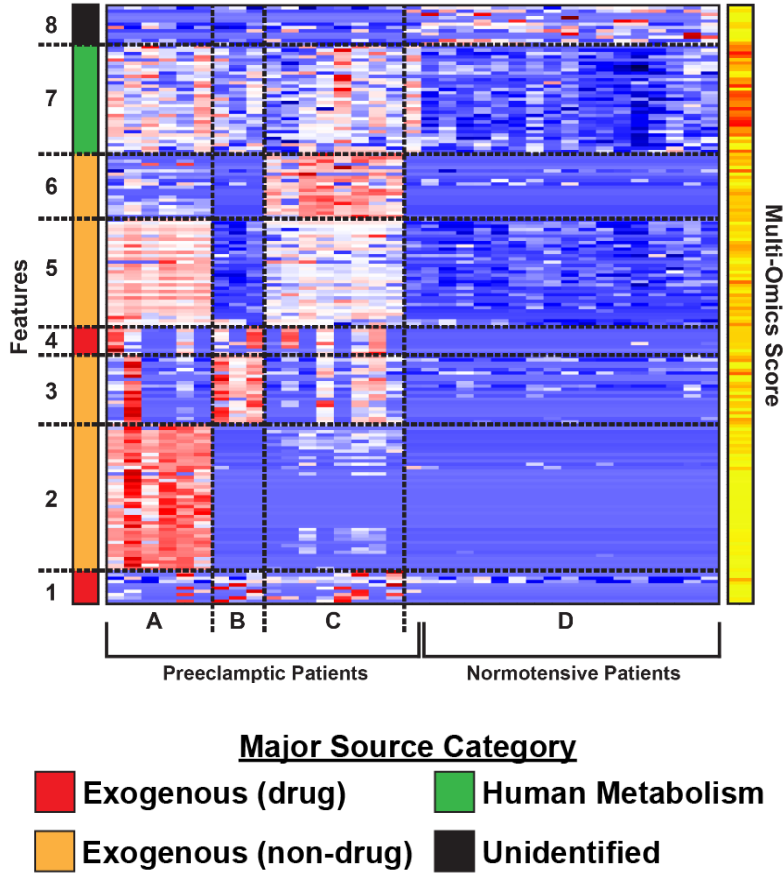
o **Endogenous metabolites** previously associated with preeclampsia (nucleotides, acyl carnitines, amino acids)

o **Exogenous drugs** known for administration with pregnancy and/or preeclampsia
  - o *Antibiotics*: cefazolin, clindamycin
  - o *Nausea*: diphenhydramine, ondansetron
  - o *Hypertension*: acetaminophen, labetalol
  - o *Steroids*: dexamethasone, betamethasone

o **Exogenous non-drugs** limited in known associations with preeclampsia
  - o *Ethanolamides/endocannabinoids*: surfactants in personal care products / neurotransmitters and receptors for biological regulation
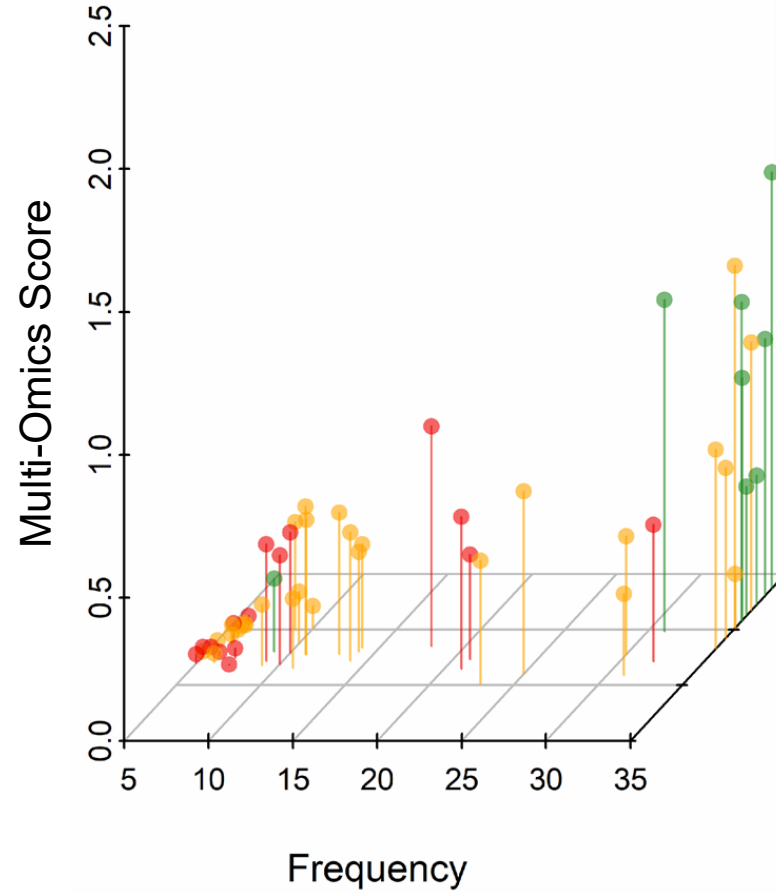
Chemical Name	Cluster	Source Category
Desmethyldiphenhydramine	1	Exogenous (drug)
Diphenhydramine		Exogenous (drug)
Formoterol		Exogenous (drug)
Dexamethasone | Betamethasone		Exogenous (drug)
Clindamycin		Exogenous (drug)
Labetalol		Exogenous (drug)
Phentermine		Exogenous (drug)
N-Linoleoylethanolamide	2	Exogenous (non-drug)
3-(1-ethyl-1-methylpropyl)-5-isoxazolamine		Exogenous (non-drug)
Methyl 5-(dimethylamino)-2-methyl-5-oxopentanoate		Exogenous (non-drug)
Undecylenoyl monoethanolamide		Exogenous (non-drug)
Methyl hydroxymethyl oleyl oxazoline		Exogenous (non-drug)
Linoleic diethanolamide		Exogenous (non-drug)
Ricinoleoyl diethanolamide		Exogenous (non-drug)
PEG (polyethylene glycol)	3	Exogenous (non-drug)
Ondansetron	4	Exogenous (drug)
Cefazolin		Exogenous (drug)
Dimethyl cyanocarbonodithioimidate		Exogenous (non-drug)
Etisomicin	5	Exogenous (drug)
Hexaminolevulinate		Exogenous (non-drug)
12-Aminododecanoic acid		Exogenous (non-drug)
Icaridin		Exogenous (non-drug)
N,N-Dimethyldodecylamine-N-oxide		Exogenous (non-drug)
Adenosine		Human metabolism
Gabapentin		Exogenous (drug)
Tyrosine		Human metabolism
Stearic acid diethanolamide | Isostearic acid diethanolamide		Exogenous (non-drug)
N-Dodecanoyl-N-methylglycine	6	Exogenous (non-drug)
2,4-Bis(1-methyl-1-phenylethyl)phenol		Exogenous (non-drug)
Acetaminophen	7	Exogenous (drug)
3,3'-[Oxybis(ethane-2,1-diyloxy)]dipropan-1-amine		Exogenous (non-drug)
N-Benzyl-N,N-dimethyldodecan-1-aminium		Exogenous (non-drug)
Lactobionic acid		Exogenous (non-drug)
Acetyl-L-carnitine		Human metabolism
3-Hydroxypentanedioic acid | Citramalic acid		Human metabolism
L-Tryptophan		Human metabolism
Valylleucine		Human metabolism
Palmitoylcarnitine		Human metabolism
Acetylcholine		Human metabolism
L-Oleoylcarnitine		Human metabolism

Chemical Feature Trends

Source Categorization of Clusters

Identified Features (Level 1-3)

All Features

Major Source Category

- Exogenous (drug)
- Exogenous (non-drug)
- Human Metabolism
- Unidentified

Preeclamptic Patients

Normotensive Patients

Multi-Omics Score

Features

Multi-Omics Score

Frequency

Abundance (Log2)

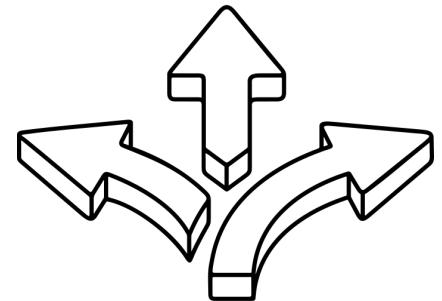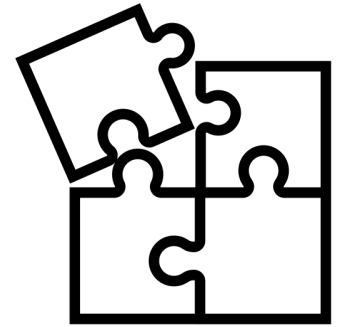- Exogenous (drug)
- Human metabolism
- Exogenous (non-drug)
- Unidentified

Summary

- Features corresponding to xenobiotics and endogenous metabolites were found associated with preeclampsia

- Integration of exposomic, epigenomic and transcriptomic analyses supports characterization chemical-biological relationships

- Evaluation of exposomic data trends can inform on exposure source pathways

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