Placental DNA Methylation Linking Exposures and Newborn Health

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Where we study methylation matters

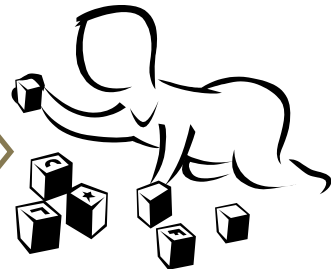
- **DNA Methylation is highly tissue specific**
- **Represents functional alteration**
- **Placenta**
  - First complex organ to form
  - Regulates intrauterine environment
  - Transport
    - Nutrients
    - Water
    - Gas
    - Waste products
  - Immuno-endocrine
    - Hormones
    - Growth factors

Role of Placental Epigenome

MATERNAL ENVIRONMENT
- Growth
- Drugs
- Nutrition
- Stress
- Exposures

PLACENTAL EPIGENOME

PLACENTAL FUNCTION



- Demonstrate placental molecular features integrate environmental signals
- Link variability in molecular features to Infant **Outcomes** (and beyond!)

Study Population: Rhode Island Child Health Study

- Hospital-based Birth Cohort
  - Moms enrolled following delivery at Women & Infant Hospital
  - WIH sees 75% of deliveries in RI
  - 2009-2014
  - Total Enrollment n=899
- Mothers
  - 18-40 years old
  - No history of psychological disorders
  - In good physical health
- Infants
  - Viable
  - No known genetic disorders
  - No life threatening illness
  - Term (≥ 37 weeks)
- Oversampled for SGA (small) and LGA (large) infants. Matched to AGA (appropriate)
  - Gestation time, infant sex, maternal age (±3 yrs)

Pregnancy → **Delivery**

Mom
- Medical Chart Review
- Questionnaire

- Maternal blood
- Toenail Samples

Infant
- Clinical Characteristics
- NNNS Assessment

- Placenta
- Cord Blood
- Toenails

Linking Molecular Character with Exposures and Outcomes

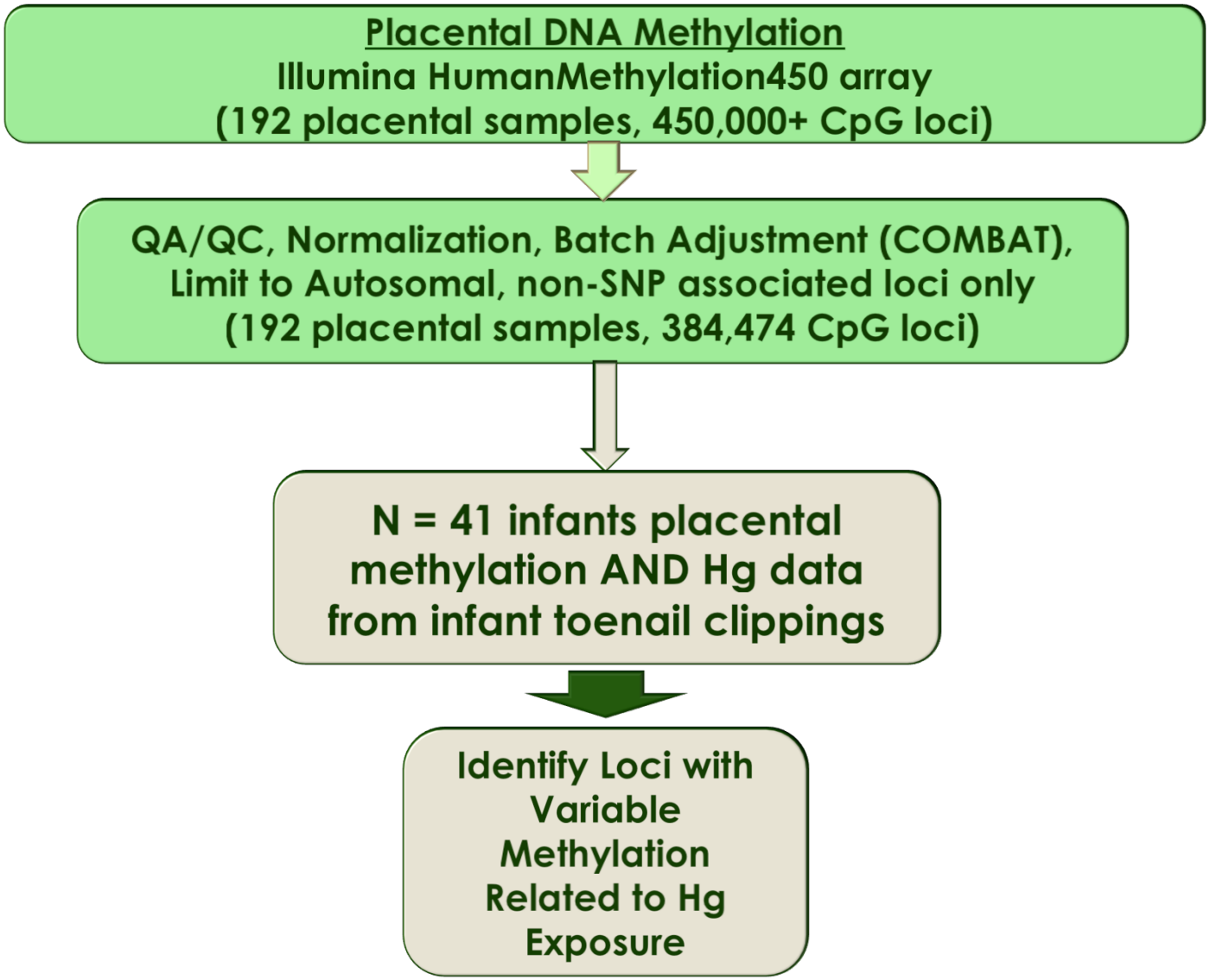
Mercury exposures in utero

- Sources of exposure
  - Dietary (fish, seafood) i.e. methylmercury (MeHg)
    - Biomethylated, biomagnified
  - Dental amalgams
  - Industrial
    - Minimata disease (Japan) (Harada et al. 1968)
    - Iraqi fungicide contamination incident (Bakir et al. 1973)
  - Air Pollution
  - Can be assessed with finger/toenail measurements (He et al. 2013; Wickre et al. 2004; Xun et al. 2013; Hinners et al. 2012)

- Crosses placenta (National Research Council 2000; Yang et al. 1997; Ilbäck et al. 1991)
  - Interferes with placental function (Boadi et al. 1992)

- Neurobehavioral effects associated with prenatal and childhood exposure
  - Infants: Cerebellum size, CNS damage, poor psychomotor development (Cace et al. 2011; Choi 1989; Llop et al. 2012)
  - Children: Memory, attention, language, visual-spatial perception (Faeroe Islands study) (Grandjean et al. 1997)

Preliminary - Discovery Study



Methylation Impacting Neuro-related Genes

- 339 CpG loci associated with infant toenail Hg
- Within genes or gene promoters associated with neurologic outcomes
  - Neurodevelopment (*DIXDC1, NRBP2, KIF26B, FEZF1, DMRTA2, ACTN1, MYO10, LRFN1*) (Singh et al. 2010; Kivimae et al. 2011; Larsson et al. 2008; Heinrich et al. 2012; Eckler et al. 2011; Shimizu and Hibi 2009; Watanabe et al. 2009; Shimizu et al. 2010; Yoshizawa et al. 2011; Konno et al. 2012; Kremerskothen et al. 2002; Silver et al. 2012; Ju et al. 2013; Raines et al. 2012; Yu et al. 2012; Morimura et al. 2006)
  - Neurobehavior (*CPLX1, LMX1B, ADD2*) (Drew et al. 2007; Glynn et al. 2007; Barreto-Valer et al. 2013; Porro et al. 2010)
  - Schizophrenia (*DIXDC1, ARVCF, MAGI2, ZIC2*) (Bradshaw and Porteous 2012; Sim et al. 2012; Mas et al. 2010; Mas et al. 2009; Chen et al. 2005)
  - ADHD (*TCERG1L*) (Neale et al. 2010; Karlsson et al. 2012; Hatayama et al. 2011)
  - Movement disorders (*NOL3, TP53INP2*) (Russell et al. 2012; Bennetts et al. 2007)
  - Autism (*PLXNA4, WNT2*) (Suda et al. 2011; Lin et al. 2012; Kalkman 2012)

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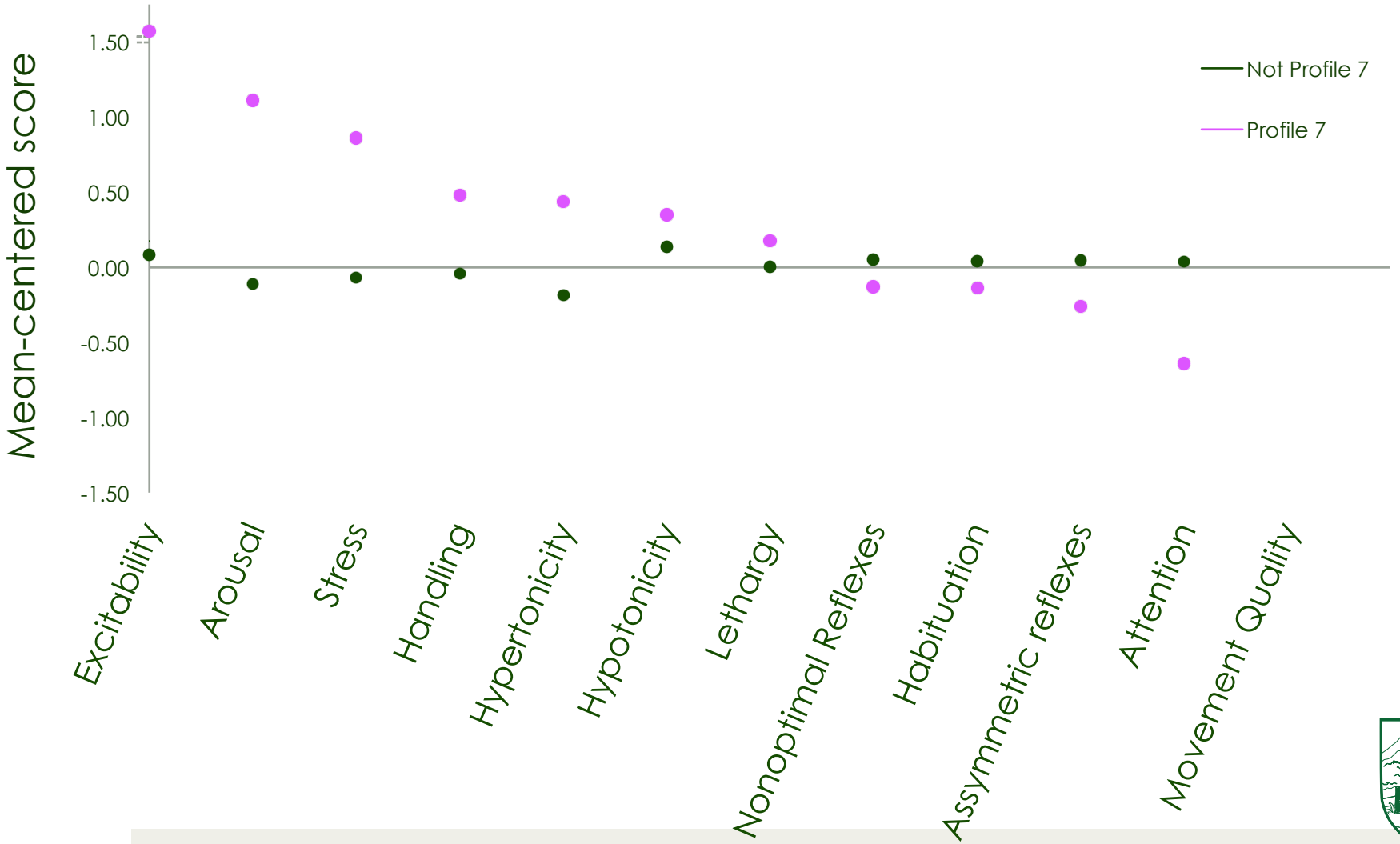
Looking at Neurobehavioral Effect

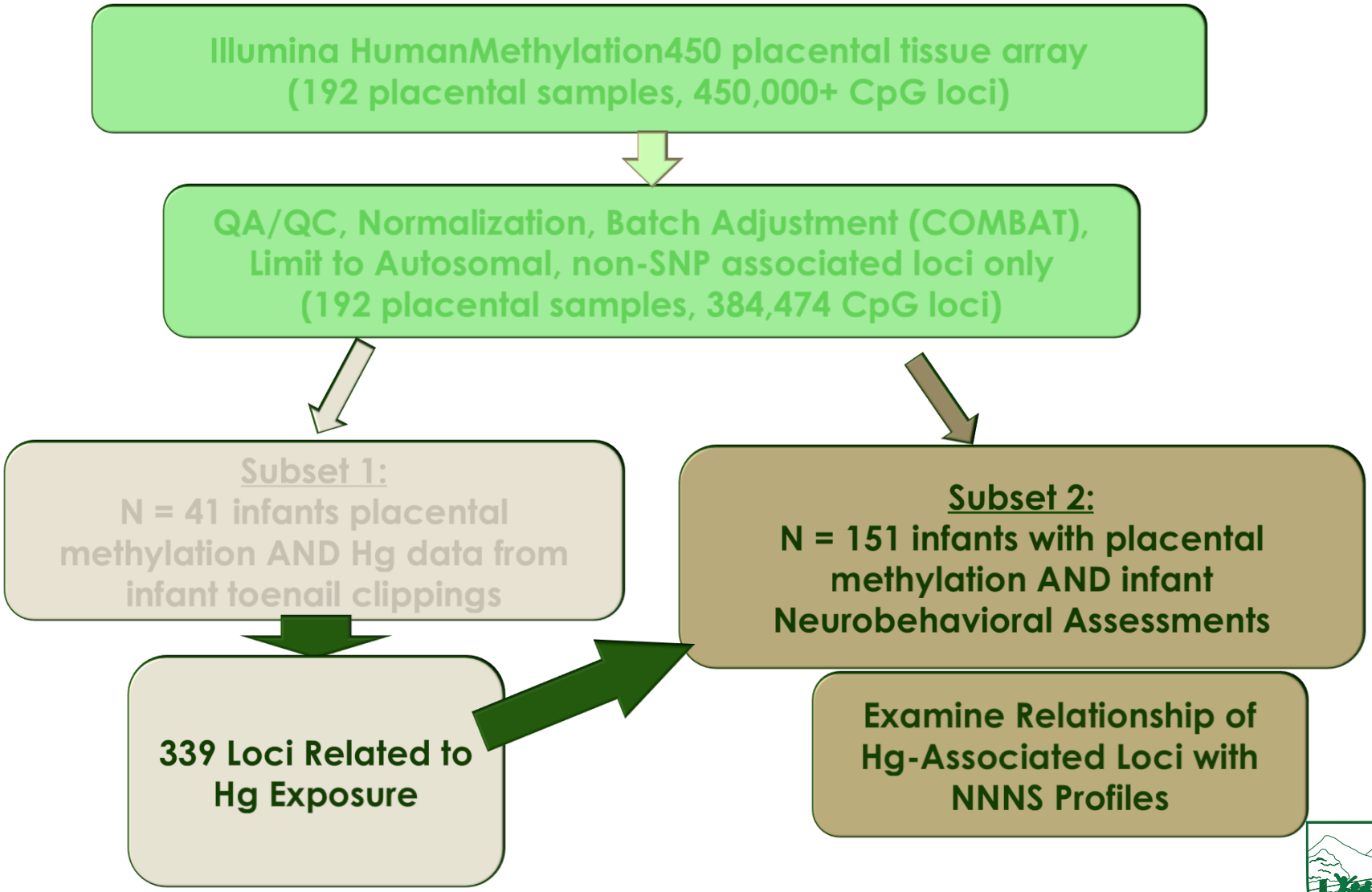
NICU Network Neurobehavioral Scales (NNNS)

- Developed by Lester and Tronick (2004)
  - Built on backbone of Neonatal Behavioral Assessment Scales (NBAS) developed by Brazelton (1973)
- Developed for use in at-risk infant
  - Specifically substance exposed – used in the Maternal Lifestyle Study
  - Designed for broad applicability
    - Generalizable
    - Reproducible
    - Sensitive to variety of infant risk factors
    - Infants from 30 weeks gestation to ~2months post-partum
- Approx. 30 minute exam performed after 24 hours of life but prior to discharge
- Examine three major area of neurodevelopment
  - Neurological
  - Behavioral
  - Stress/Abstinence
- Summarized into 13 Summary Scales reflecting various aspects of neurodevelopment
  - Can examine individually
  - Used latent profiling strategy to create profiles of neurobehavior (Liu et al Pediatrics 2010)

Focused on "At-risk" profile



Discovery Scheme



6 CpG Hg-associated loci are associated with NNNS High Excitability Profile

Illumina CpG Designation	Gene Symbol	P Value	UCSC CpG Island Designation
cg13267931	***EMID2***	8.25×10^{-6}	**Island**
cg14874750	***EMID2***	6.06×10^{-5}	**Island**
cg23424003	***EMID2***	7.30×10^{-5}	**Island**
cg27179533	***EMID2***	5.46×10^{-5}	**Island**
cg27528510	***EMID2***	9.00×10^{-5}	**Island**
cg14048874	***EMID2***	0.0023	**Island**
cg14175932		2.84×10^{-5}	
cg17128947	***CPLX1***	0.0054	**Island**
cg25385940	***TTC23***	0.0059	**N Shore**
cg10470368		0.0075	

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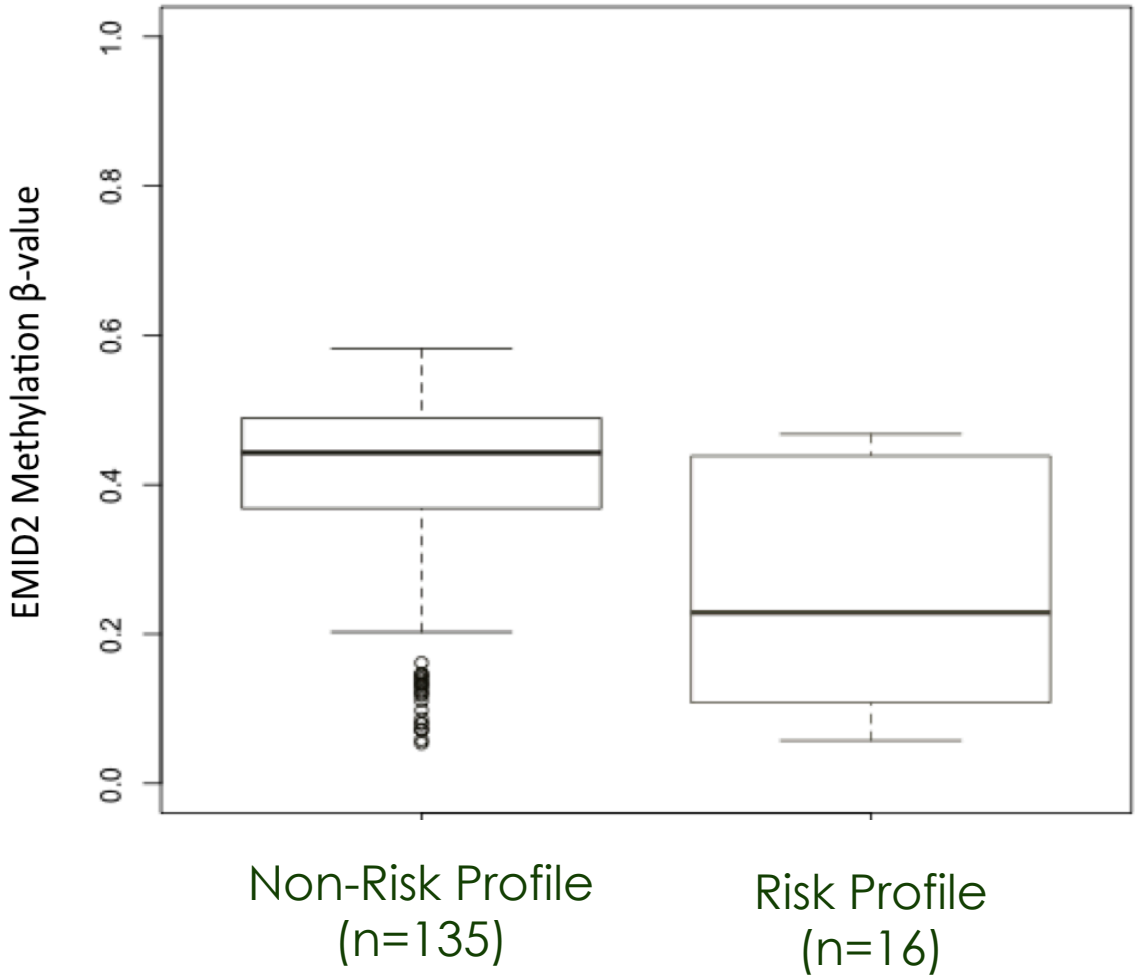
Placental hypomethylation of *EMID2* associated with Risk Behavioral Profile



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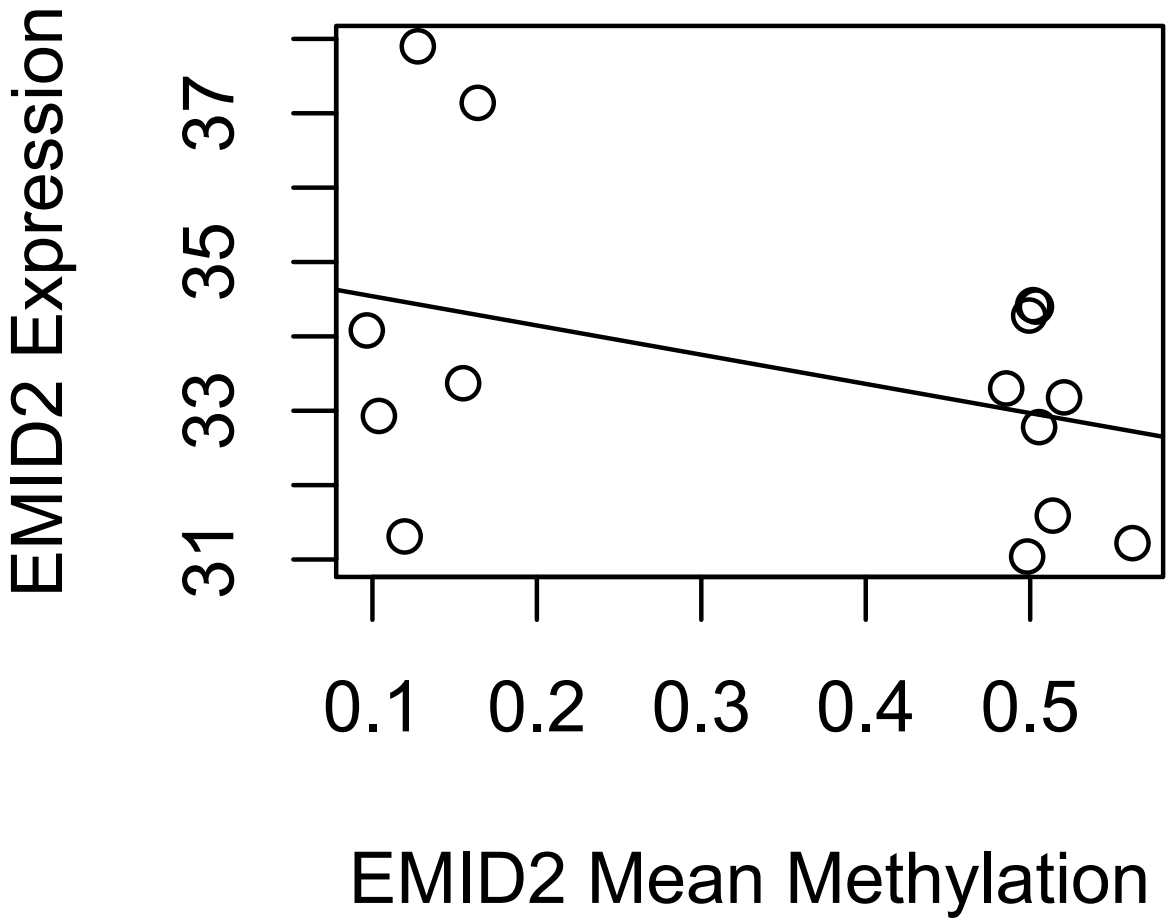
Methylation Correlated with Expression



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EMID2

- Collagen protein, unknown placental function
- Variant associated with asthma/airway hyperresponsiveness in nasal passages (Pasaje et al. 2011; Pasaje et al. 2012)
- A SNP within EMID2 mediates side effects on vision and hearing in response to an antidepressant (Adkins et al. 2012)
- More work needed to understand the functional role of this gene in placenta

DNA Methylation as Toxicant Mechanism

- Alterations to genes or pathways can have long-term consequences on development
- DNA methylation is susceptible to environmental signals
  - Toxicant Exposures
  - Maternal Factors/Lifestyle
  - Stress, Psychosocial adversity
- Can then link altered DNA methylation to critical outcomes
- Ongoing work
  - Better defining what environment can do
  - Consequences of these alterations – Various Health Outcomes

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