Newborn Anthropometry and Differential DNA Methylation and Gene Expression in Human Placentas

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Background

- Fetal growth in utero is affected by genetic predisposition & environmental factors
- Infants born IUGR (intrauterine growth restriction), SGA (small for gestational age) and LGA (large for gestational age) are at risk for both short term and long term sequelae
Long-term sequelae of SGA, IUGR and LGA

IUGR

- Suboptimal maternal-fetal nutritional environment
  - Maternal nutrient restriction, gestational diabetes, maternal obesity
  - Programming
  - Cellular energy metabolism
    - Glucose handling, lipid metabolism, mitochondrial biogenesis
      - ↑ Storage  ↓ Oxidation
      - Critical windows: organogenesis
  - Adipose tissue
    - Excess lipid storage
    - Lipolysis
    - ER stress
  - Muscle
    - Lipid accumulation
  - Liver
    - Cholestasis
    - Lipid accumulation
  - Pancreas
    - Impaired glucose sensing
    - Hyperinsulinism

LGA

- Microvascular system
  - Fetomaternal metabolism
  - Lipid accumulation
- Brain
  - Insulin resistance and/or type 2 diabetes
- Hypertension
- Hyperglycemia
- Hyperinsulinism
- CVD
- Obesity
The role of the placenta in developmental programming of disease

Maternal diet

Maternal metabolism

Maternal body composition

Maternal endocrinology

Maternal environment

PLACENTA
- Nutrient transport
- Hormone synthesis/degradation
- Vascular resistance

Fetal responses to maternal environment
- Epigenetic modification of gene expression
- Blood flow redistribution
- Altered pattern of fetal growth
Epigenetics refers to heritable, reversible forms of gene modification that function independently of the DNA sequence. DNA methylation is an example of epigenetic modification, in which patterns of methylation of DNA nucleotides can modify gene expression.
Objectives

(1) To investigate the association of newborn anthropometry (IUGR or LGA) on changes in placental DNA methylation and gene expression

(2) To identify novel clinically relevant pathways that may be involved in the developmental programming of cardiovascular and metabolic disease in humans

(3) To validate the functional importance of identified pathways in an animal model
Methods

Human placental samples (>37 wks GA)
   n: IUGR=6, AGA=6, LGA=5

DNA isolated

Reduced Representation Bisulfite Sequencing

Data analysis/Pathway analysis

Gene expression validation by QT-PCR
DNA methylation profiling (RRBS)

For each sample:
- 46M reads with mapability of ~56% and sequencing depth of 13.3X
- Provides estimation of methylation level from 0 (unmethylated) to 100 (fully methylated) on ~3.6M CpG sites enriched in CpG islands
Global methylation levels

Placentas from pregnancies associated with IUGR are hypermethylated compared to AGA fetuses, and placentas associated with LGA are hypomethylated compared to pregnancies resulting in IUGR fetuses.
Differentially methylated regions

(Left) Methylation patterns of DMR show unique signatures for each birthweight group.

(Above) In general, DMRs are more abundant in promoters and 3’ UTR and depleted in coding regions and intergenic regions.
We identified 1015, 906, and 1022 DMRs, corresponding to 191, 171, and 172 genes that show clear differential methylation in comparisons between AGA versus IUGR, AGA versus LGA, and IUGR versus LGA.
To identify specific loci whose methylation level shows a strong association with offspring anthropometrics, we calculated covariance between methylation levels at each cytosine and the selected phenotype from each sample.
Risk for adult metabolic and cardiovascular disease

**Metabolic**
- **SLC36A1** is a gene encoding a member of proton-assisted amino acid transporters, important in promoting normal growth.
- Various studies have shown crosstalk between glucose and fat processing pathways and these transporters.

**Endocrine**
- **PTPRN2** encodes a major autoantigen seen in insulin-dependent diabetes mellitus.

**Cardiovascular**
- **CASZ1** encodes a transcription factor that may function as a tumor suppressor.
- Single nucleotide polymorphisms in this gene have been associated with blood pressure variation.

**Inflammation**
- **IL10** is an important cytokine in pregnancy that promotes immune tolerance.
- Low levels of IL10 have been associated with recurrent miscarriage, IUGR and preeclampsia.
- It has also been associated with hypertension in animal models.

Multiple DMR in an intron of **SLC36A1** are hypomethylated in LGA compared to AGA.

DMR within the genebody demonstrate found DMRs with variable hyper/hypomethylation pattern.

Both IUGR and LGA groups were hypermethylated in the last exon, compared to AGA.
Validation of gene expression

(A) SLC36A1 mRNA

(B) PTPRN2 mRNA

(C) CASZ1 mRNA

(D) IL10 mRNA

* p ≤ 0.05
  * p ≤ 0.02
  * p ≤ 0.04
  * p ≤ 0.03
IL-10 is a potent anti-inflammatory cytokine that acts as a pleitropic regulator of immune tolerance in pregnancy.
Maternal caloric restriction results in intrauterine growth restricted pups. The placenta of these pups demonstrate degenerative vascular and trophoblastic changes.
RNA sequencing and validation

**AUTOPHAGY**
- DRAM1: p<0.0001
- FBXO32: p<0.0001
- SCD1: p<0.007

**ER STRESS**
- PDI4: p<0.0001
- CRELD2: p<0.05
- DERL3: p<0.002

**VASCULAR INFLAMMATION**
- MAP3K6: p<0.02
- C1Qa: p<0.001
- IL10: p<0.001
Conclusions

1. Placental hypermethylation is seen in pregnancies associated with human IUGR, and relative placental hypomethylation seen in pregnancies associated with LGA infants.

2. There are a number of genes important in cardiovascular, metabolic, and immunologic pathways that demonstrate differential methylation patterns based on neonatal anthropometrics.

3. Patterns of DNA methylation are associated with altered gene expression.
Discussion and Future Directions

• Discovery-based studies on epigenetic mechanisms such as this provides a gateway to identify mechanisms critical to the developmental programming of adult disease in IUGR and LGA infants.

• Future studies are planned in our animal model to establish causal relationships between alterations in pathways regulated by IL10 and vascular and trophoblast remodeling and placental insufficiency in IUGR.
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