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



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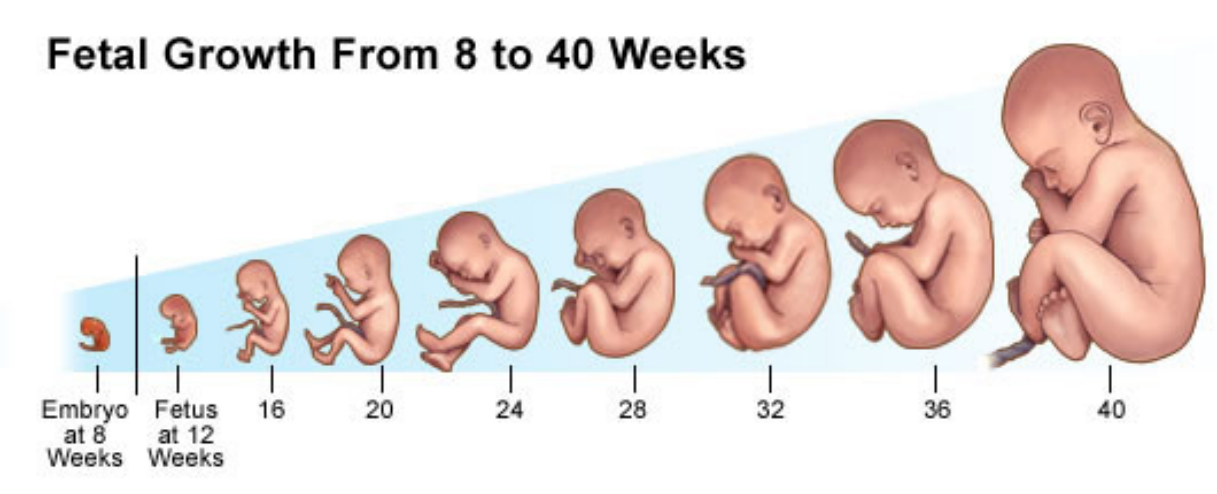
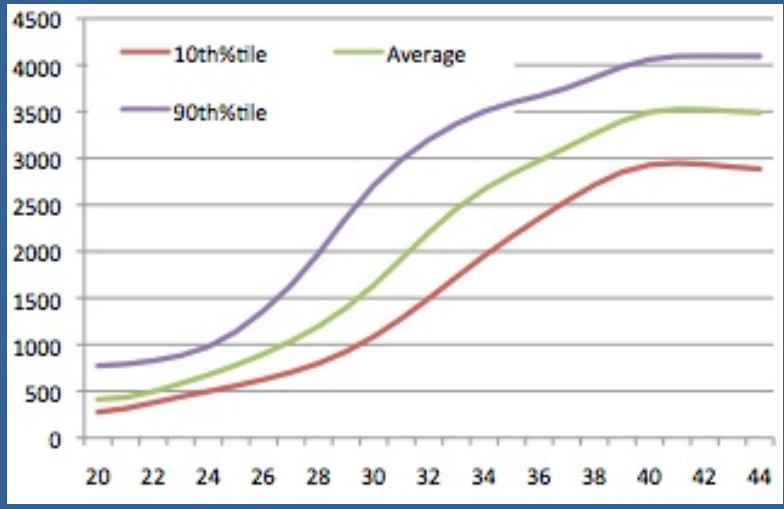
Background

- 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

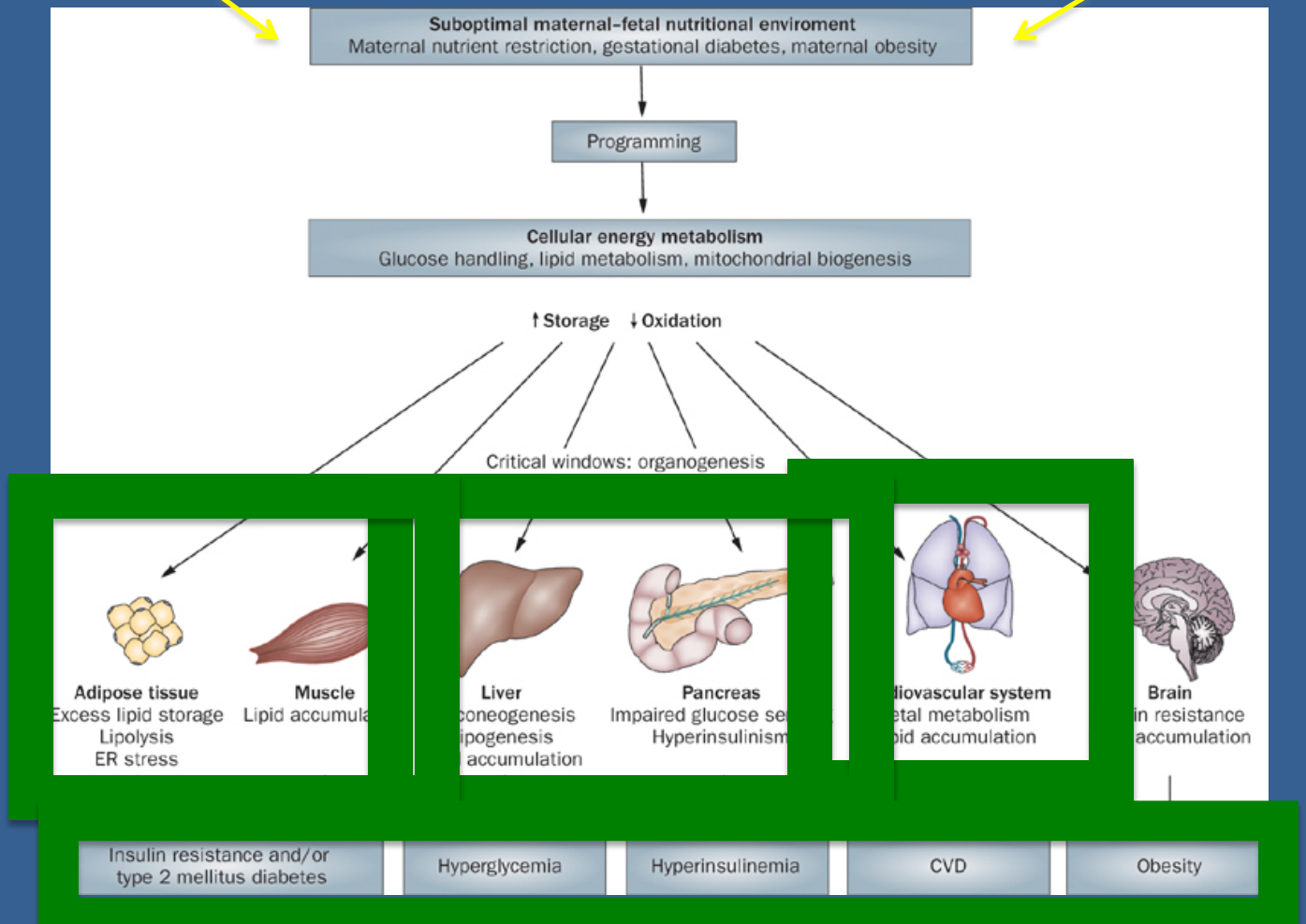




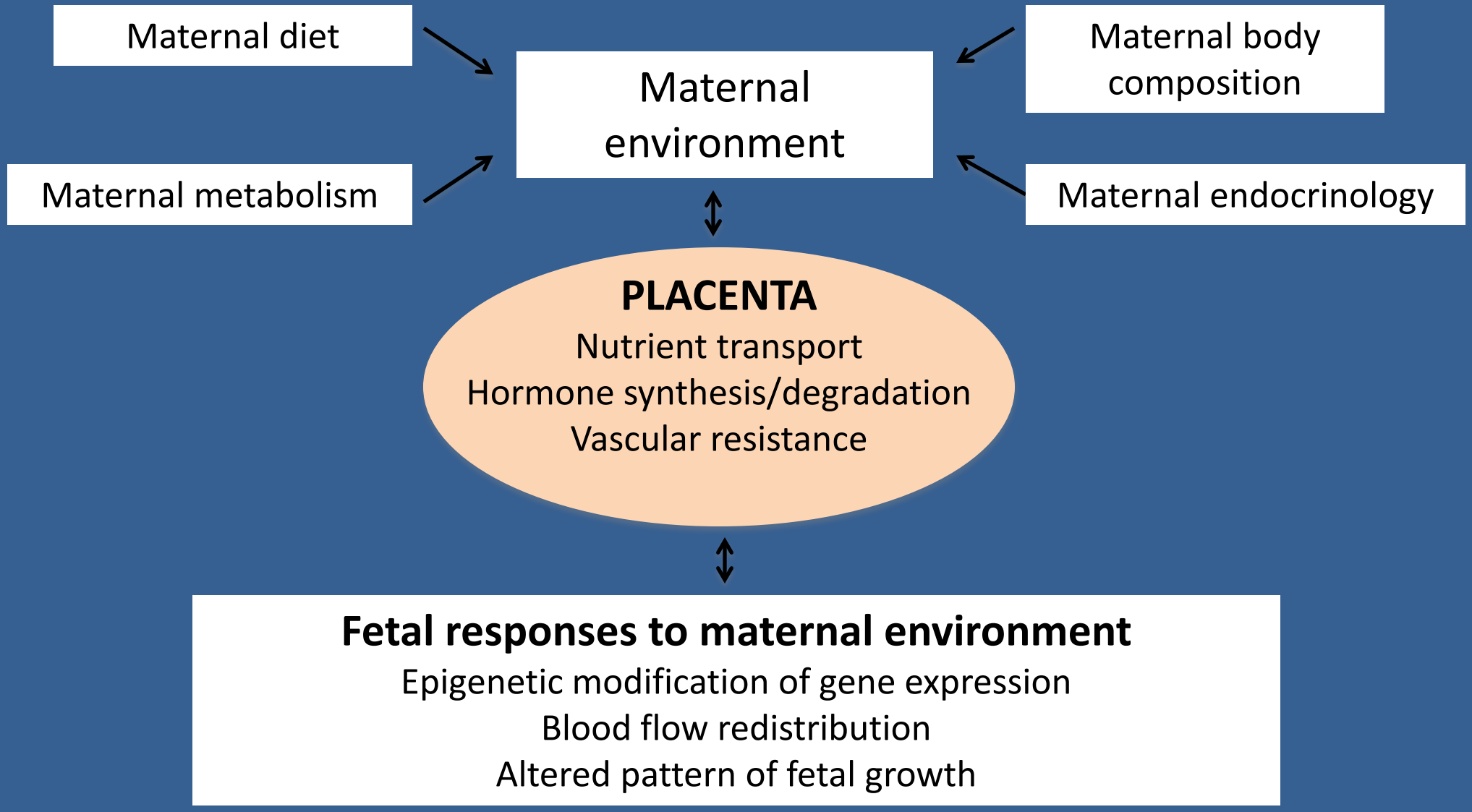
Long-term sequelae of SGA, IUGR and LGA

IUGR

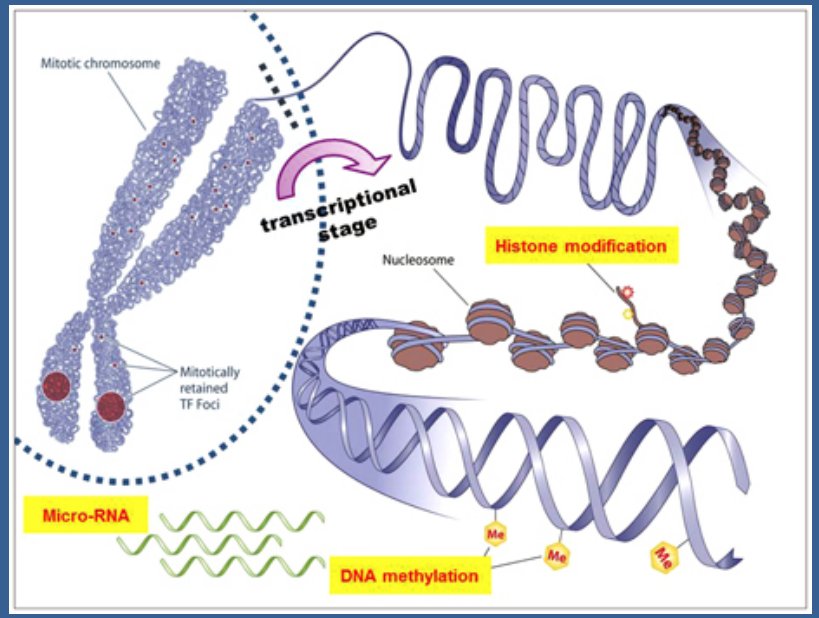
LGA

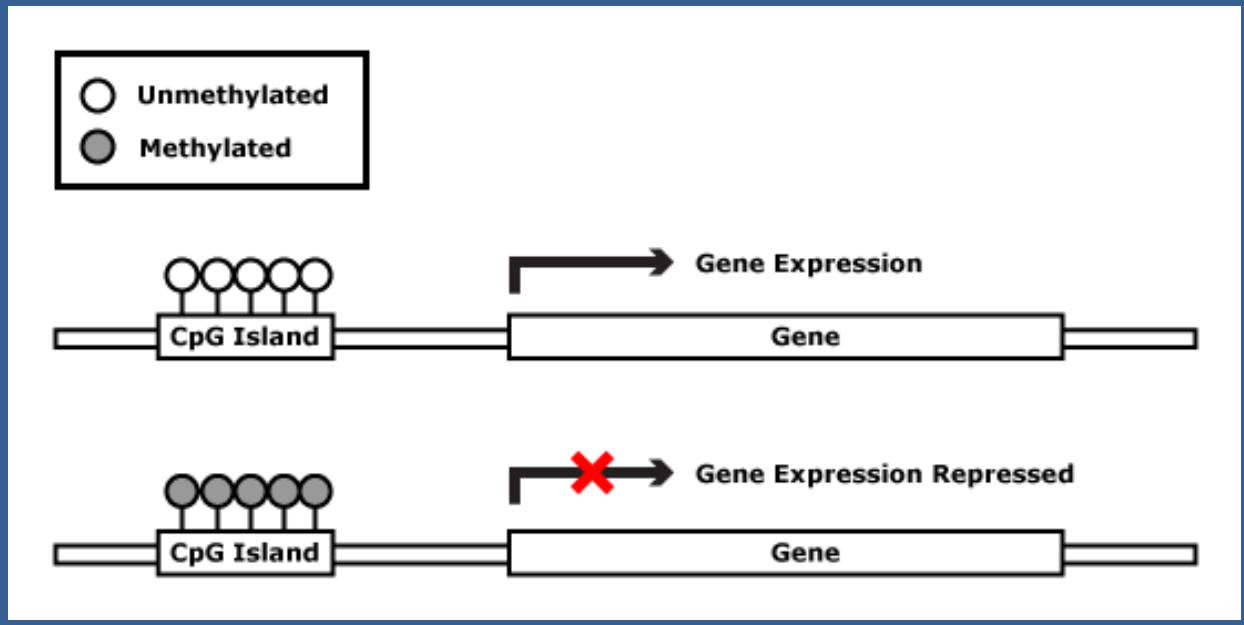


The role of the placenta in developmental programming of disease



Epigenetics





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

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Data analysis/Pathway analysis

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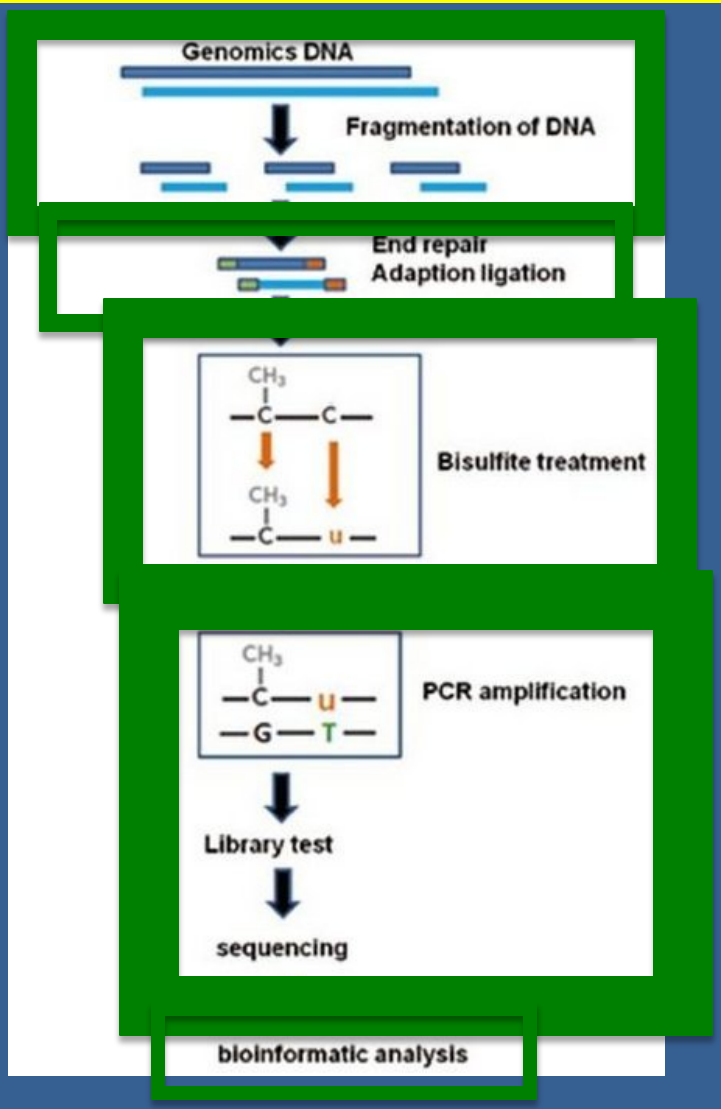
Gene expression validation by QT-PCR

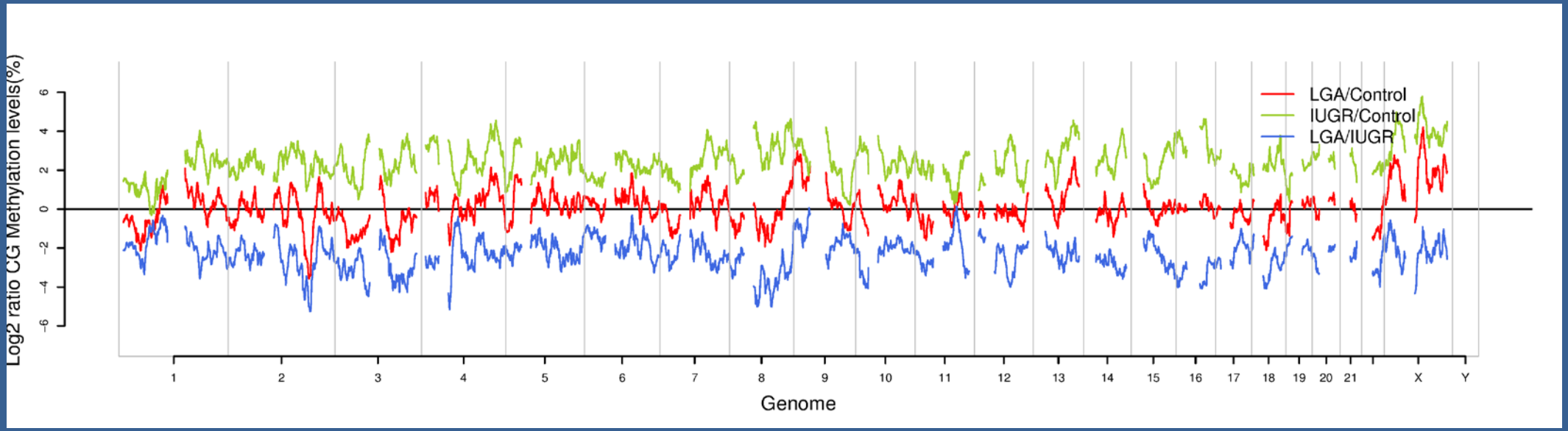
DNA methylation profiling (RRBS)



For each sample:

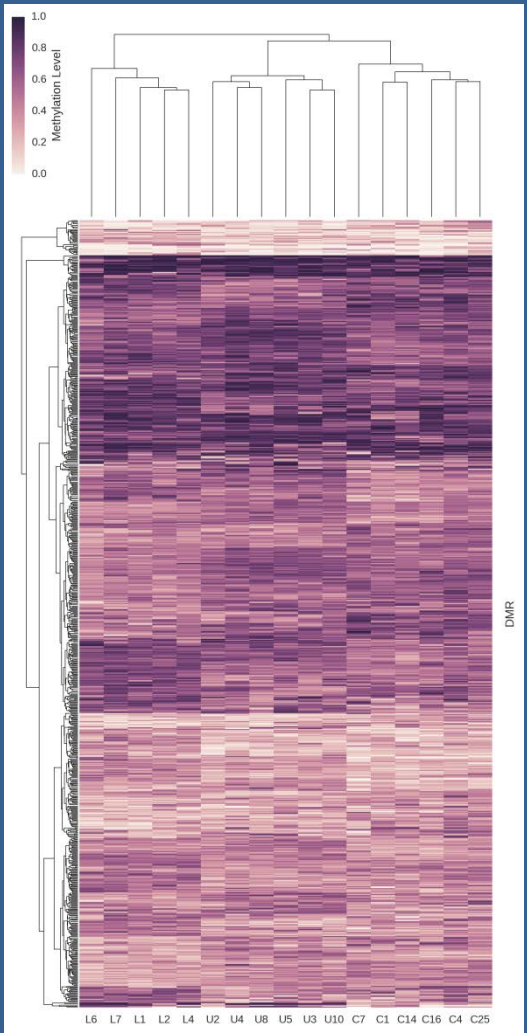
- 46M reads with mapability of ~56% and sequencing depth of 13.3X
- Provides estimation of methylation level from 0 (unmethylated) to 100 (fully methylated) on ~3.6M CpG sites enriched in CpG islands

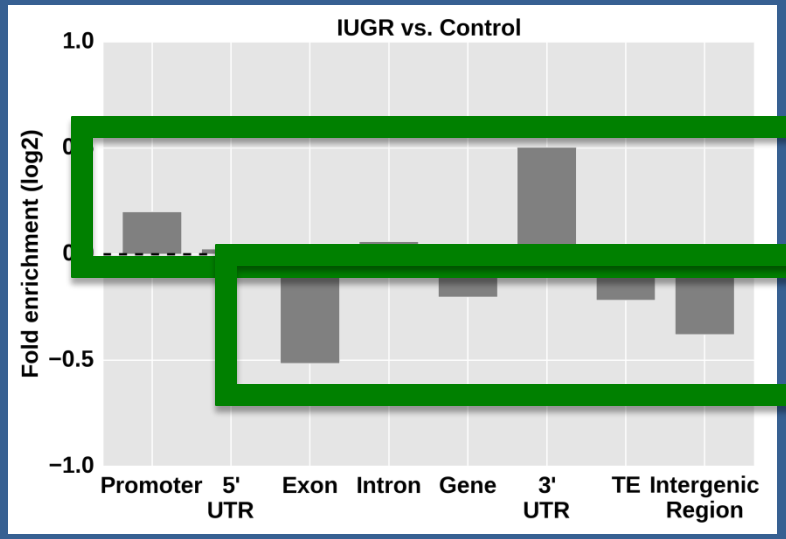
Global methylation levels

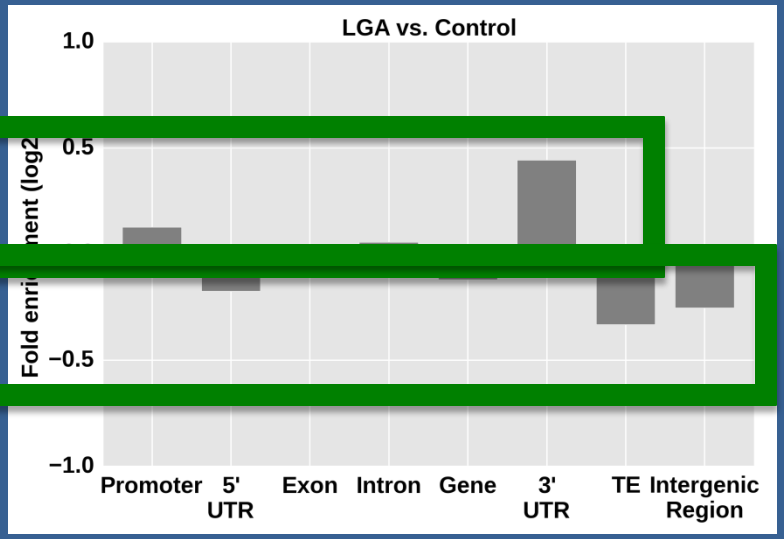


Placentas from pregnancies associated with IUGR are hypermethylated compared to AGA fetuses, and placentas associated with LGA are hypomethylated compared to pregnancies resulting in IUGR fetuses.

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.

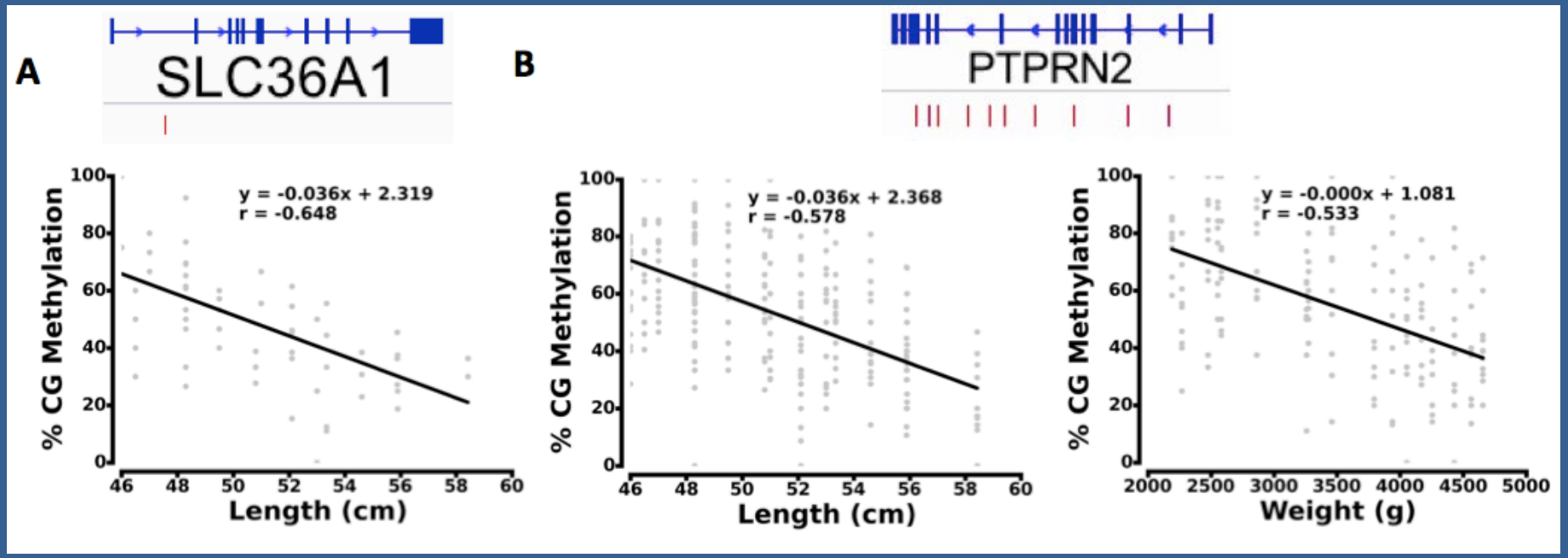
Differentially methylated genes

Table 2. Diff[illegible]nd the enriched gene networks

Comparison	#DMR	#Differentially Methylated Genes	Top Major Networks [Significance score = -log10(*P* value)]
Control vs. IUG[illegible]	1015	191	Cell-to-cell signaling and interaction, cellular assembly and organization, nervous system development and function (52) Connective tissue disorders, dermatological diseases and conditions, hereditary disorder (37) Cell-to-cell signaling and interaction, cellular assembly and organization, nervous system development and function (37) Lipid metabolism, small molecule biochemistry, Cell-to-cell signaling and interaction, cellular assembly and organization, nervous system development and function (33) Cardiac hypoplasia, cardiovascular disease, developmental disorder (32)
Control vs. LGA	906	171	Connective tissue disorders, dermatological diseases and conditions, hereditary disorder (38) Cancer, organismal injury and abnormalities, reproductive system disease (37) Connective tissue development and function, embryonic development, organ development (32) Connective tissue disorders, inflammatory disease, skeletal and muscular disorders (32) Cellular assembly and organization, cellular function and maintenance, cell death and survival (32)
IUGR vs. LGA	1022	172	Cell morphology, cellular assembly and organization, cancer (42) Neurological disease, cell morphology, organismal injury and abnormalities (33) Nervous system development and function, tissue morphology, embryonic development (31) Connective tissue disorders, dermatological diseases and conditions, behavior (31) Small molecule biochemistry, cancer, neurological disease (31)

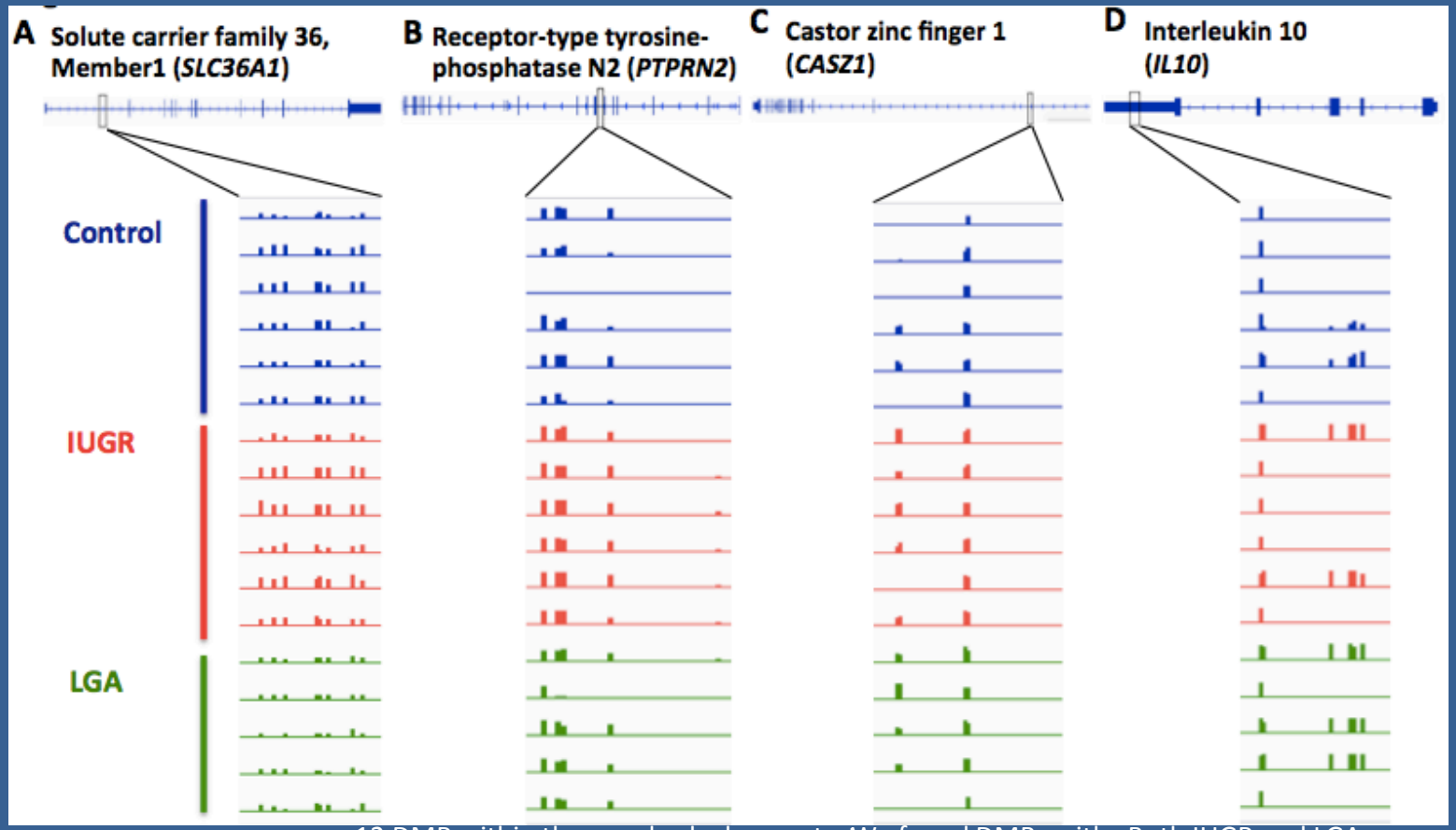
We identified 1015, 906, and 1022 DMRs, corresponding to 191, 171, and 172 genes that show clear differential methylation in comparisons between AGA versus IUGR, AGA versus LGA, and IUGR versus LGA.

Covariance analysis



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



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

12 DMR within the genebody demonstrate variable hyper/hypomethylation pattern in LGA and IUGR

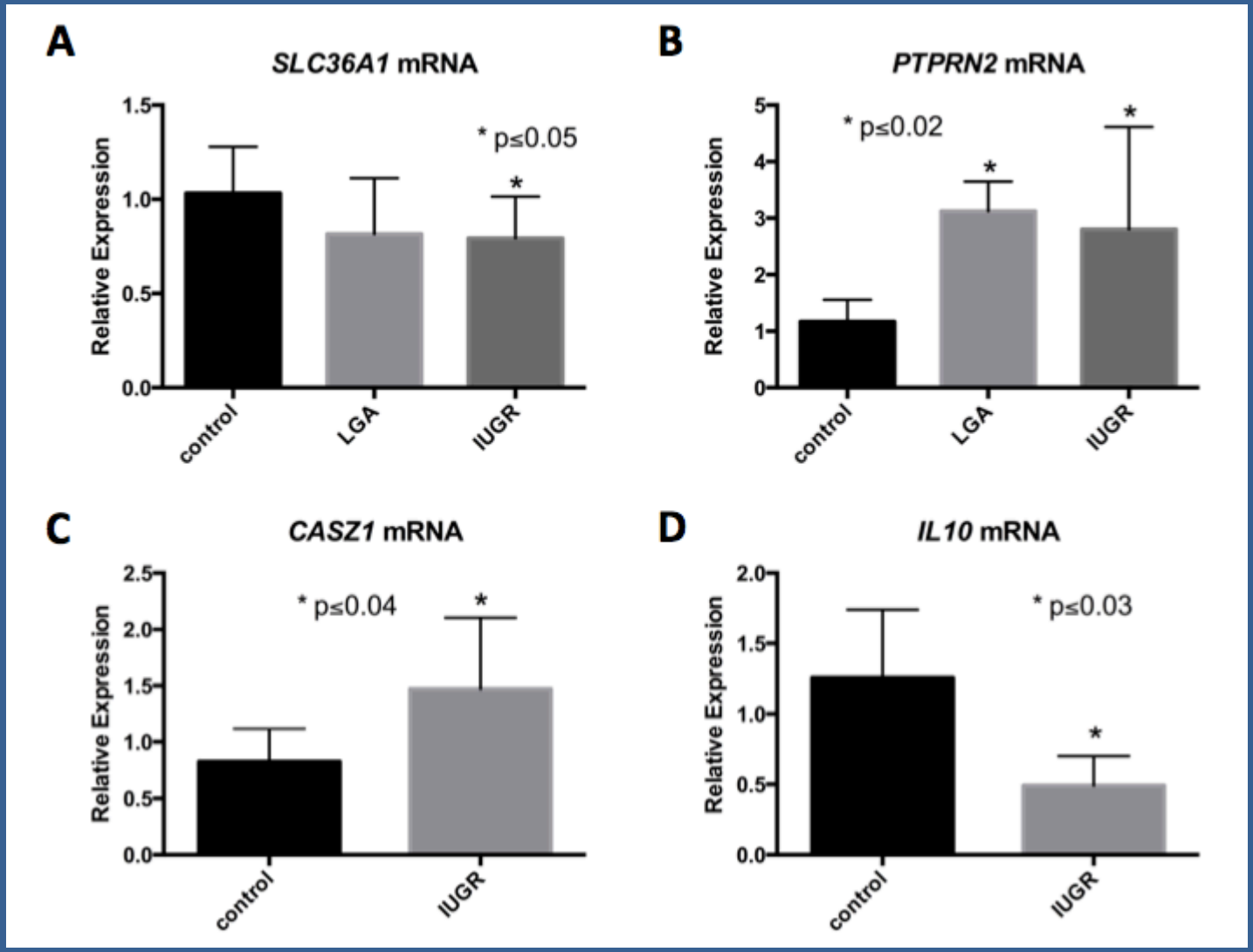
We found DMRs with hypermethylation in IUGR compared to AGA

Both IUGR and LGA groups were hypermethylated in the last exon, compared to AGA

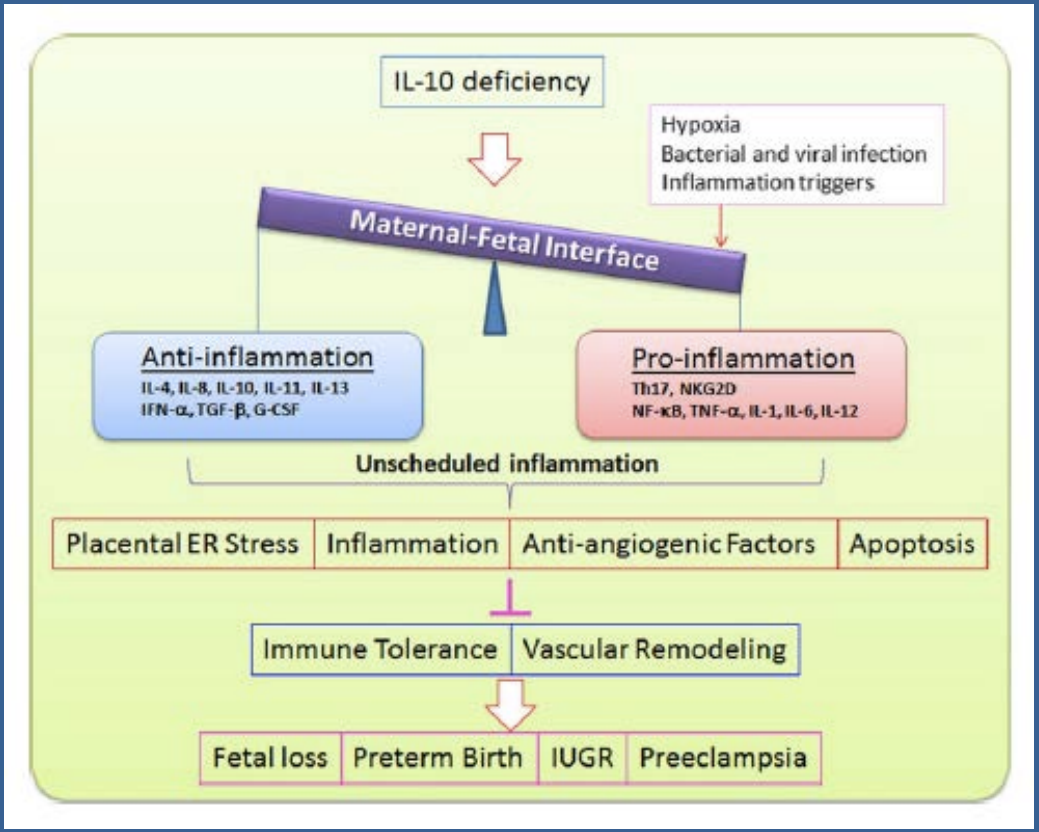
Inflammation

- *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.

Validation of gene expression

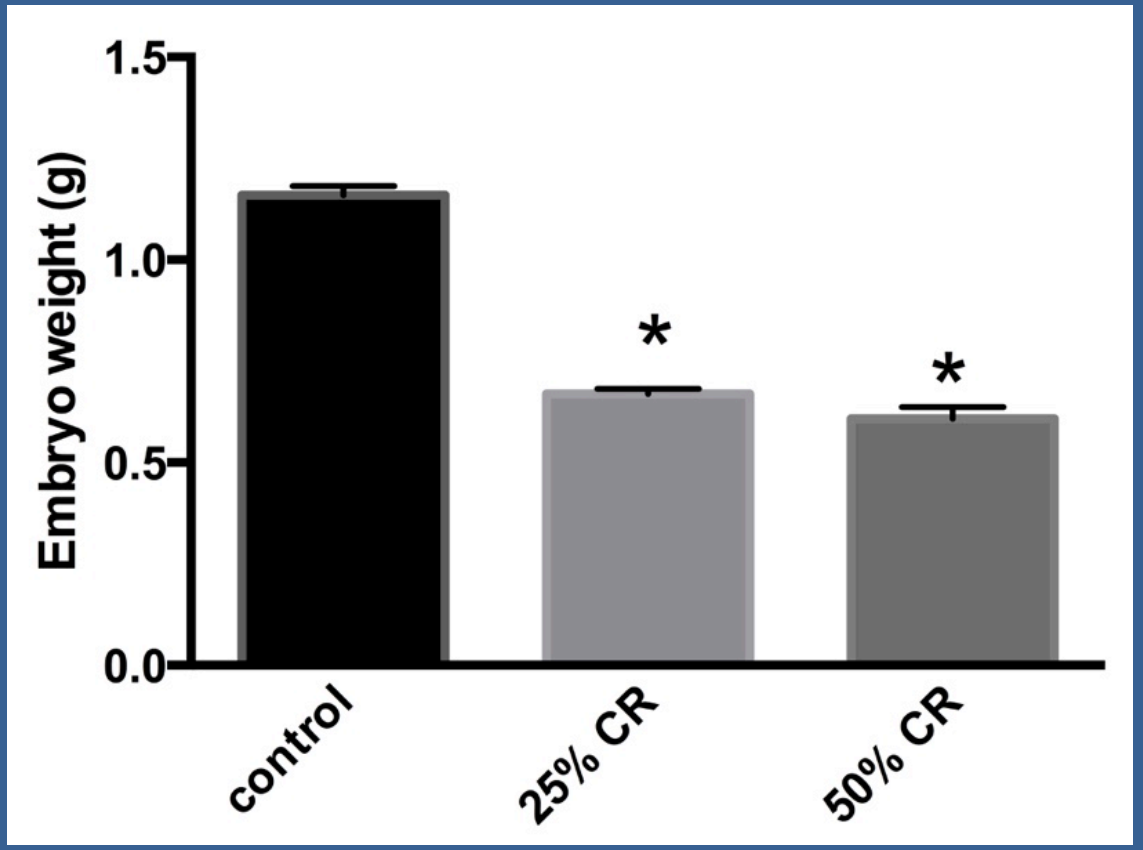


IL-10



IL10 is a potent anti-inflammatory cytokine that acts as a pleitropic regulator of immune tolerance in pregnancy.

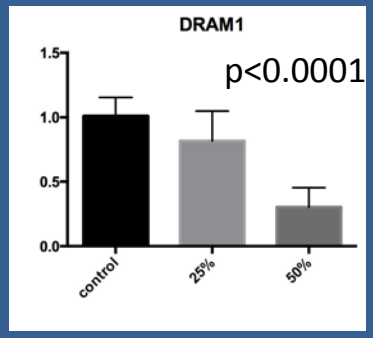
Animal model of IUGR resulting from maternal caloric restriction

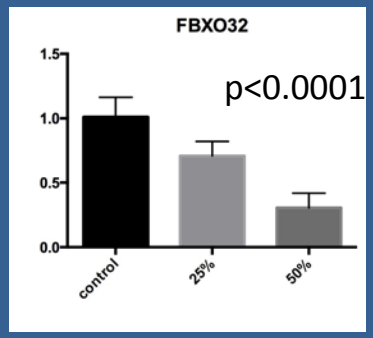


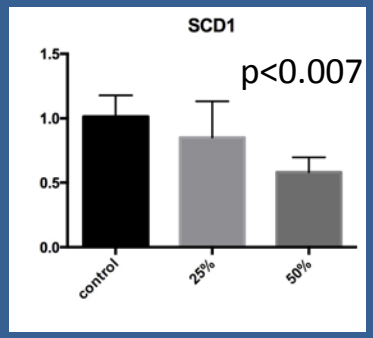
Maternal caloric restriction results in intrauterine growth restricted pups. The placenta of these pups demonstrate degenerative vascular and trophoblastic changes.

RNA sequencing and validation

AUTOPHAGY







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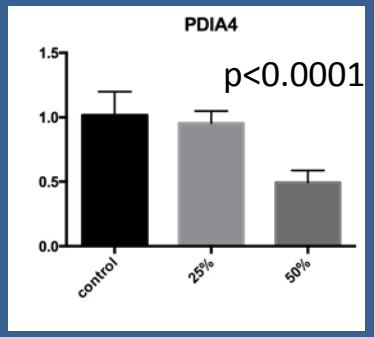
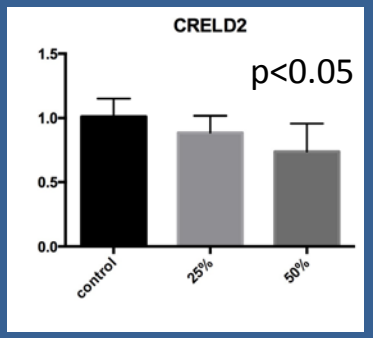
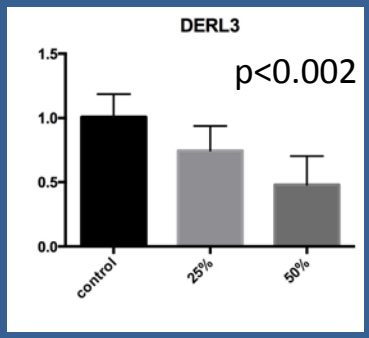
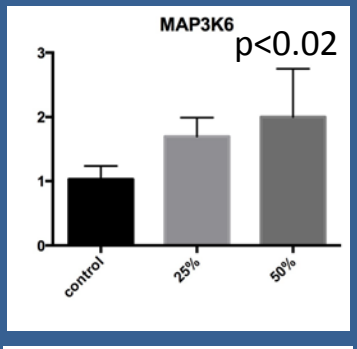
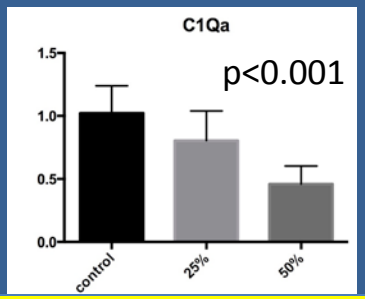


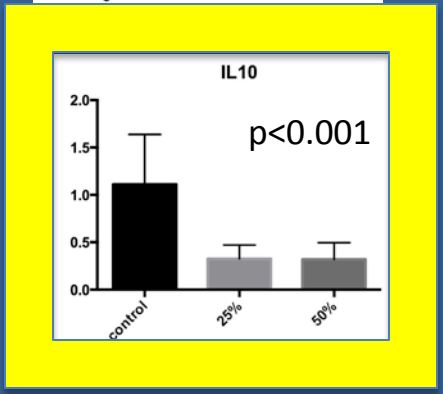




VASCULAR INFLAMMATION







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.

THANK YOU

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