DNA Methylation as a Marker of the Intra Uterine Environment

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ABSTRACT

The placenta functions not only as a conduit for nutrient and waste exchange between mother and fetus, but also as a regulator of the intrauterine environment. Recent work has identified changes in the expression of candidate genes, often through epigenetic alteration, which alter the placenta's function and impact fetal growth. Using the Illumina Infinium HumanMethylation27 BeadChip array, we examined genome-wide DNA methylation patterns in 206 term human placenta samples. Semi-supervised recursive-partitioned mixture modeling was employed to analyze data from a training series of placenta samples and identify methylation profiles associated with aberrant fetal growth. A number of CpG loci were found to effectively differentially classify intrauterine growth restriction (IUGR) and small for gestational age (SGA) placentas from appropriate for gestational age (AGA) placentas, and these associations were validated in a masked testing series of samples. Our work demonstrated that patterns of DNA methylation in human placenta are reliably and significantly associated with infant growth and serve as a proof of principle that methylation status in the human term placenta can function as a marker for the intrauterine environment.

BACKGROUND AND CLINICAL RELEVANCE

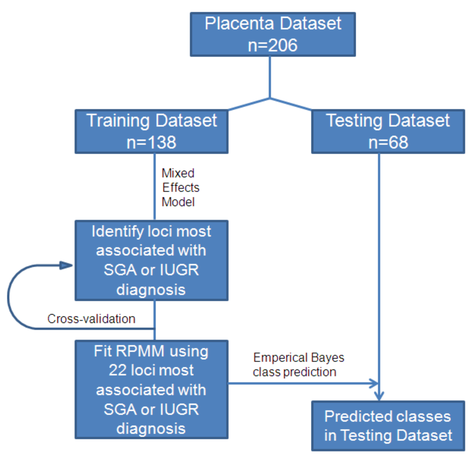
- Altered DNA methylation without genetic mutation is capable of altering gene expression resulting in phenotypic change (Waterland, R.A., and Jirtle, R.L., 2003) and has been associated with the development of cancerous tumors (Bird, A., 2002).
- Changes in DNA methylation patterns can decrease mammalian implantation success (Rahnama, F., et.al, 2006; Rahnama, F., et.al 2009) and have been implicated in other high risk pregnancy states (Novakovic, B., 2009; Yuen, R.K.C., et.al 2009).
- The alterated DNA methylation profiles also affect the fetus resulting in lower birth weights (Serman, L., et.al, 2007)
- Epigenetic alterations in specific genes have been linked to preeclampsia and intrauterine growth restriction (Bourque, D.K., et.al. 2010; Tabano, S. et.al. 2010)

SPECIFIC AIMS

- Determine a specific profile of differentially methylated genes in placental tissue that classify samples according to growth restriction.
- Explore the biology of these genes and elucidate the role of the placenta in fetal development.

METHODOLOGY AND STATISTICAL ANALYSIS

- DNA from 206 human term-placenta samples were bisulfite modified and tested using the Illumina DNA HumanMethylation27 BeadChip array to determine the methylation status of 14,495 genes.
- The genes most associated with growth restriction were identified on a training sample subset and validated on an independent testing subset.

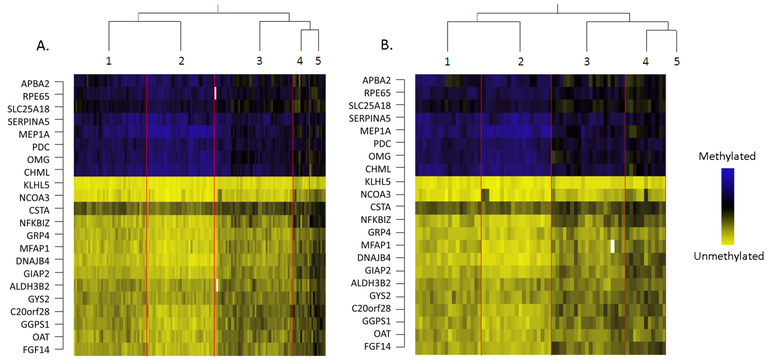


Flow chart demonstrating the statistical handling the complete dataset. The full dataset was split randomly into the training and testing subsets stratified by infant gender and gestational age.

DEMOGRAPHICS

	Total n=206	Training n=138	Testing n=68
Growth Status			
AGA, n (%)	117 (56.8)	79 (57.2)	38 (55.9)
SGA or IUGR, n(%)	89 (43.2)	59 (42.8)	30 (44.1)
Infant Birth Weight in grams, mean (SD)	2937.4 (599)	2951.6 (596)	2908.7 (609)
Infant Gender, n (%)			
Females	99 (48)	66 (47.8)	33 (48.5)
Males	107 (52)	72 (52.2)	35 (51.5)
Infant Gestational Age in weeks, mean (SD)	38.2 (2.0)	38.3 (1.8)	38.0 (2.3)
Maternal Age in years, mean (SD)	27.9 (5.9)	27.6 (5.7)	28.5 (6.3)

METHYLATION PROFILES ARE ASSOCIATED WITH INFANT GROWTH STATUS



	Class 1	Class 2	Class 3	Class 4	Class 5	Total
AGA n(%)	19 (24)	13 (16)	33 (42)	7 (9)	7 (9)	79 (100)
SGA or IUGR n(%)	21 (36)	24 (41)	10 (17)	2 (3)	2 (3)	59 (100)

	Class 1	Class 2	Class 3	Class 4	Class 5	Total
AGA n(%)	8 (21)	8 (21)	11 (29)	11 (29)	0 (0)	38 (100)
SGA or IUGR n(%)	10 (33)	11 (37)	9 (30)	0 (0)	0 (0)	30 (100)

Fig A. Heatmap of the 138 placenta samples in the training data (columns) clustered according to the methylation status of the 22 loci selected by the mixed effects model and cross validation procedures (rows) with demographic classification of SGA or IUGR diagnosed totals and total number of samples per class in the table below the heatmap. Fig B. Heatmap of the 68 placenta samples in the testing data clustered into 4 of the 5 categories defined in the training data with demographic classification of SGA or IUGR diagnosed totals and total number of samples per class in the table below the heatmap.

SPECIFIC METHYLATION PROFILES ARE ASSOCIATED WITH GROWTH RESTRICTION CONTROLLED FOR CONFOUNDERS

		Odds Ratios (95% CI) of being SGA or IUGR	p-value
Classes 3-5, n (%)	9 (30)	Reference	
Classes 1-2, n (%)	21 (70)	**2.94 (1.05, 7.38)**	**0.045**
Infant Gender n (%)			
Male	35 (51)	Reference	
Female	33 (49)	0.90 (0.32,8.73)	0.834
MaternalAge in years, mean (SD)	28.5 (6.3)	1.00 (0.91,1.08)	0.908
Delivery Method n (%)*			
Vaginal	43 (65)	Reference	
C-Section	23 (35)	0.65 (0.21, 1.92)	0.445

* missing data on 2 samples

Multi variable analysis of the association between SSRPMM class and SGA or IUGR classification.

GENE SET ENRICHMENT ANALYSIS

TFBS Pathway	PvalueGSEA	PvalueWilcox
NFE2	0.024	0.05
CEBPB	0.033	0.031
FOXO4	0.037	0.035

Entry	KEGG Pathway	PvalueGSEA	PvalueWilcox
hsa05016	Huntington's disease	0.002	0.002
hsa05010	Alzheimer's disease	0.021	0.006
hsa03020	RNA polymerase	0.017	0.026
hsa03060	Protein export	0.028	0.028
hsa03440	Homologous recombination	0.033	0.05

RESULTS AND FUTURE DIRECTIONS

- 22 loci were found to be most associated with infant growth status.
- These 22 loci can produce a model-based classification pattern, which when applied to an independent sample of data can correctly classify the samples with growth restriction.
- This suggests that we have identified those loci which are robustly associated with infant growth.
- GSEA analysis reveal association with TFBS: NFE2, C/EBP beta, and FOXO4 and several pathways involved in neurological disorders.
- Genes will be validated by pyro-sequencing.

CONCLUSIONS

- Our findings suggest that coordinate epigenetic alterations contribute to infant growth status.
- The genes identified suggest that in the placenta, developmental and cell cycle control pathways are critical regulators of infant outcome.
- The changes in these genes may alter the trajectory of infant growth.

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