Consequences of folate depletion during development for DNA methylation and gene expression in the fetal mouse

Jill McKay¹, Dianne Ford¹, Caroline Relton¹, Chris Evelo², Michiel Adriaens² & John Mathers¹

¹Human Nutrition Research Centre, Institute for Ageing and Health, Newcastle University
²BiGCaT, Maastricht University
Developmental Origins of Health & Disease

Nutritional Insults in utero

Altered Programming

Adulthood

Increased disease risk

e.g. Waterland & Jirtle, Mol & Cell Biol, 2003

e.g. Lillycrop et al 2005, BJN

e.g. Sinclair et al 2007, PNAS
Epigenetic Mechanisms

- Candidate mechanisms for developmental programming
- Established *in utero*
- Modifiable by environmental factors
- Influence gene expression

Folate

- Metabolic role in transfer of 1-carbon units
- Important in cellular pathways for:
  - amino acid interconversion
  - nucleic acid biosynthesis
  - methylation of biological molecules
- Essential for normal development
- Low folate intake during pregnancy is associated with increased risk of NTDs
Hypothesis

Offspring of folate depleted mothers have altered gene expression caused via DNA methylation changes
Folate Depleted Mouse Model

- **Mating D0**
- **Gestation**
- **Sampling D17.5**

2 weeks → 4 weeks → Gestation

- **Control Diet**
  - Lab Chow
  - Control diet: 2 mg/kg Folic acid
- **Low Folate Diet**
  - Low folate diet: 0.4 mg/kg Folic acid

Hepatic RNA assessed for gene expression changes
Hepatic DNA methylation assessed
Gene Expression Arrays

Male Liver Tissue

RNA & DNA extraction

RNA Quality check

Shipped to Service XS

Affymetrix whole genome mouse array
  n = 6/group
Methylated DNA Immunoprecipitation (MeDIP) for Methylation Arrays

1. Genomic DNA
   - DNA fragmentation by sonication & heat denaturing
   - Immunoprecipitation with 5-methylcytidine Ab

2. Input DNA Cy3 labelled
3. MeDIP DNA Cy5 labelled

4. Hybridised to CpG Island and Promoter arrays

Symbols:
- Methylated CpG
- Unmethylated CpG
Array Analysis

BiGCaT MeDIP & ChIP analysis pipeline
(Adrieens, 2011, manuscript in preparation)

DNA methylation microarrays → Raw data preprocessing → Identify enriched regions → ANOVA to identify differential methylation

Transcriptomics microarrays → Raw data preprocessing → Factorial design: statistical analysis

ArrayAnalysis.org
(Eijssen, 2011, manuscript in preparation)

Figure composed by Michiel Adriaens

Poster: ‘Consequences of folate depletion during development and high fat intake from weaning on adiposity, gene expression and DNA methylation in adult mice. ’
Gene expression changes in response to low maternal folate

- Fold change $>1.2$ or $<-1.2$
- Significant $P$ value $<0.05$

![Gene expression changes](chart.png)

- Down Regulated: 434 genes
- Up Regulated: 572 genes
DNA methylation changes in response to low maternal folate

- Significant P value <0.05
Genes with altered expression & DNA methylation

72 genes were differentially expressed AND differentially methylated in response to low maternal folate

\[ y = -0.2038x + 0.4256 \]
\[ R^2 = 0.0115 \]

Expected line of best fit if ↑ methylation was associated with ↓ expression
Changes in gene expression of 33 genes were potentially caused by changes in DNA methylation: DNA methylation ↑, gene transcription ↓
Indirect Effects

Changes in gene expression of 39 genes were caused by other mechanisms: altered expression of transcription factors, histone modifications or post-transcription microRNA events.

- *73 TFs (5.8%) significantly altered expression*

- **↑ Hist1h4i** & - Histone family
- **↑ Hist3h2ba**
- **↑ Hira** - Histone chaperone
- **↓ Hdac8** - Histone deacetylase
Conclusions

• Maternal low folate alters both gene expression & DNA methylation in fetal liver

• More genes were hypermethylated than hypomethylated in response to low folate

• 72 genes were differentially expressed AND methylated in response to low maternal folate

• Suggests mechanisms other than DNA methylation are involved in observed altered gene expression e.g. changes in transcription factor expression, histone modifications etc

• It is essential to consider both direct and indirect effects when integrating epigenomic and transcriptomic data
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