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Comparative Genomics

Altered Hippocampal DNA Methylation and Gene Expression Patterns in an Iron Deficiency Pig Model of Cognitive Development

Schachtschneider Kyle,[1,2] Rund Laurie A.,[1] Madsen Ole,[2] Johnson Rodney W.,[1] Groenen Martien A.,[2] Schook Lawrence B.,[1]

[1]Department of Animal Sciences, [3]Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Illinois, USA
[2]Animal Breeding and Genomics Center, Wageningen University, Wageningen, The Netherlands

Introduction:

•Iron deficiency is a common worldwide childhood nutrient deficiency, and has been linked to cognitive impairments.

•Altered DNA methylation levels are associated with aberrant gene transcription and represent a link between genetics and environmental signals that has been reported to play an important role in human pathologies including cancer and neurological disorders, revealing the importance of accessing DNA methylation patterns in understanding disease development.

•Hippocampus samples (3 Severely Iron Deficient (SID), 4 Control) used in this study were collected from female Yorkshire piglets from a previously published study assessing the effects of iron deficiency on spatial learning and memory in a neonatal pig model of human infants (Figures 1 & 2; Rytych et al., 2012).

•Piglets were fed sufficient (6.30 mg FE/kg body weight) or deficient (0.630 mg Fe/kg body weight) concentrations of Iron.

- Iron Deficiency was confirmed via weekly hematocrit and hemoglobin measurements

•At 19 days of age piglet spatial learning and memory assessment began using a clear plastic T-maze with visual cues.

- SID piglets took longer to locate the reward, had fewer correct choices, and did not improve their performance over time

Figure 1: T-maze Used in Cognitive Development Experiments

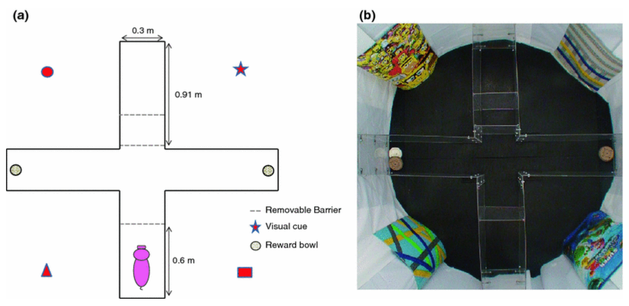
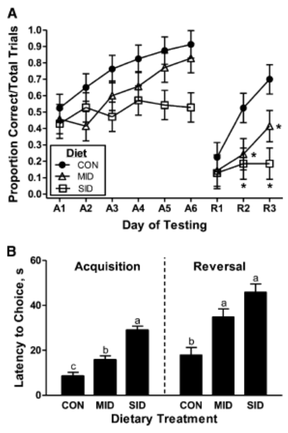
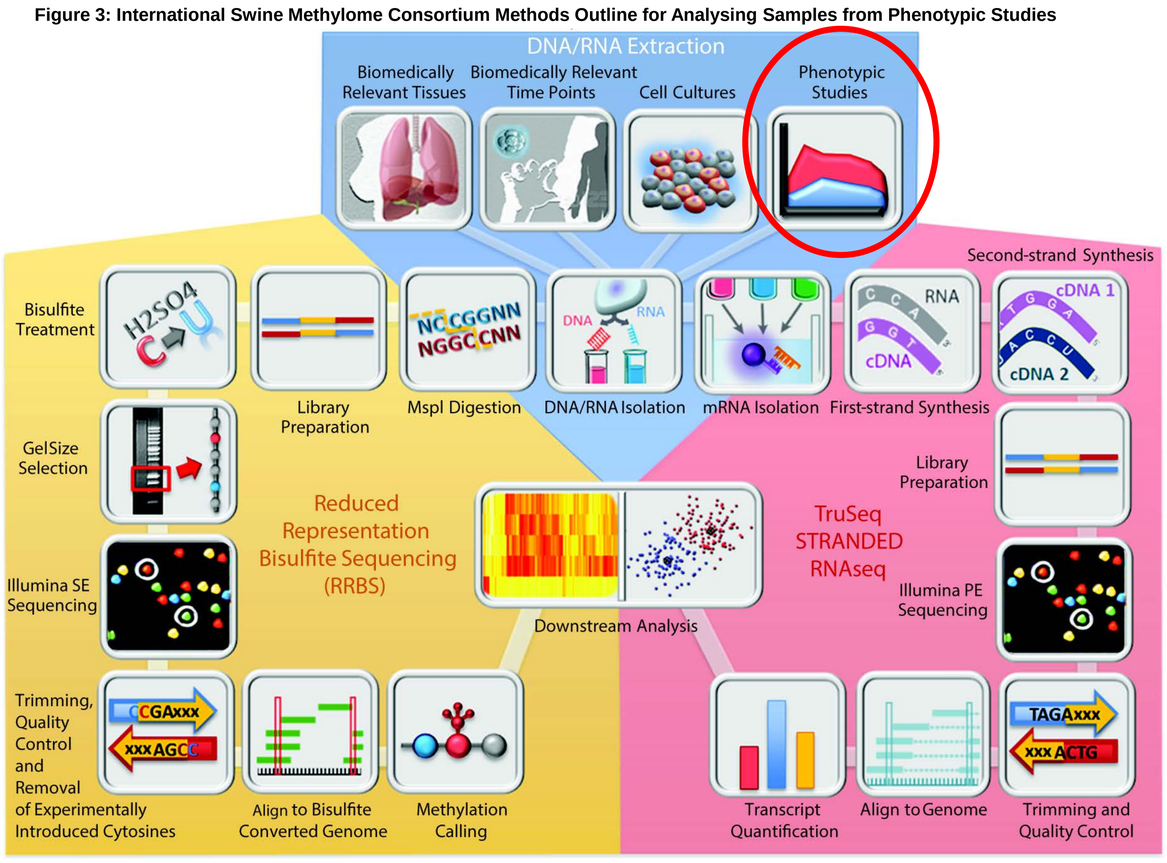


Figure 2: Piglet Performance During the Acquisition Phase (A1-6) and Reversal Phase (R1-3) of Testing for T-maze Task



Materials and Methods:

Figure 3: International Swine Methylome Consortium Methods Outline for Analysing Samples from Phenotypic Studies



- Piglets were euthanized at 29 days of age
  - DNA and RNA was extracted from hippocampus samples
- RRBS and RNA-seq performed on Illumina HiSeq 2000
- RRBS Analysis:
  - 30 – 160 bp fragments selected
  - Alignment and methylation calling performed using BSseeker2 (Guo et al., 2013)
  - Differential methylation analysis was performed on all sites with a minimum of 10 reads/site and 25% methylation difference using the R package methylkit (Akalin et al., 2012)
- RNA-seq Analysis:
  - Alignment performed using Tophat2 (Kim et al., 2013)
  - Differentially expressed gene (DEG) analysis performed using Cufflinks (Trapnell et al., 2013)

Results:

Table 1: RRBS Coverage Statistics

Sample	Group	Total # Reads	Uniquely Aligned	Genome Coverage	Average Coverage	Average Methylation	CpG Sites Minimum 1X Coverage	CpG Sites Minimum 10X Coverage
13	Control	53,541,305	58.84%	1.37%	22.25	40.63%	2,299,474	752,148
19	Control	54,675,673	60.36%	1.25%	30.02	41.37%	2,097,676	860,642
25	Control	53,948,120	59.07%	1.35%	27.32	43.19%	2,256,644	945,878
31	Control	56,712,021	60.06%	1.50%	24.42	43.08%	2,470,448	889,235
15	SID	33,017,887	58.69%	1.14%	17.47	41.29%	1,967,887	662,453
33	SID	36,519,483	59.29%	1.27%	20.03	42.52%	2,167,135	841,584
27a	SID	46,815,672	58.82%	1.27%	24.78	42.28%	2,145,740	873,773
Theoretical Max				1.75%				2,812,047

Figure 2: Average Number of CpG Sites within Genomic Locations

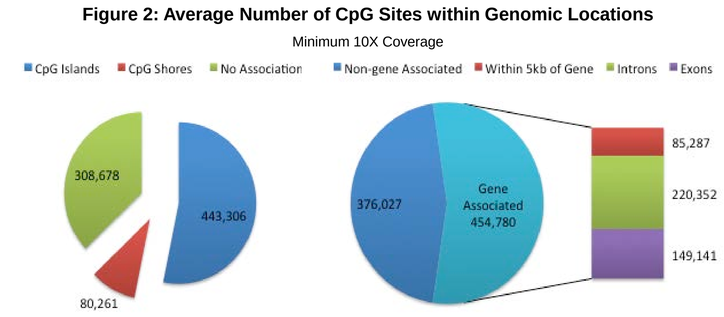


Table 2: Differential Methylation Results Between SID and Control Hippocampus

Differentially Methylated Regions (DMR; 100 bp)	Differentially Methylated Sites (DMS)	Hypomethylated DMR	Hypermethylated DMR	Hypomethylated DMS	Hypermethylated DMS	Differentially Expressed Gene-Associated DMR	Differentially Expressed Gene-Associated DMS
346	454	193	153	238	216	3	6

Table 3: DEGs Involved in Learning, Memory, and Iron Transport

Gene	Expression	Description
PRSS12	Decreased	Promotes structural reorganizations associated with learning and memory operations. Causes mental retardation when truncated in humans.
NETO1	Decreased	NETO1 knockout mice show signs of reduced spatial learning and memory.
KCNQ5	Decreased	Important in the regulation of neuronal excitability, reduced function associated with schizophrenia
PAK3	Decreased	Mutations linked to mental retardation.
TF	Increased	Iron binding transport protein responsible for the transport of Iron to sites of storage and utilization.
TTYH1	Increased	Believed to be involved in the transport of iron across cell membranes.

Figure 4: DEGs Between SID and Control Hippocampus

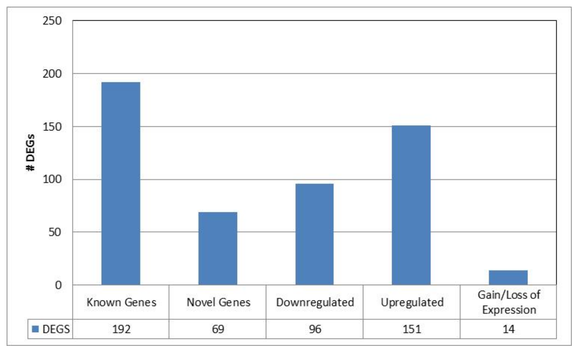
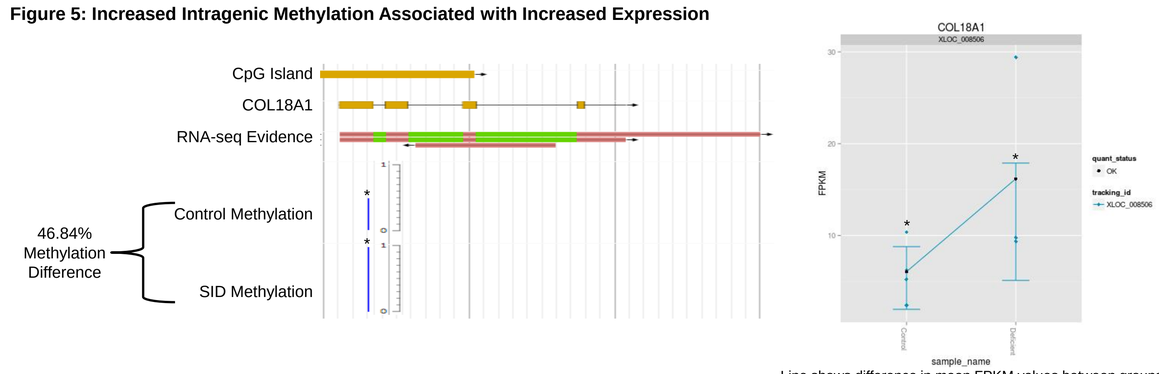


Figure 5: Increased Intragenic Methylation Associated with Increased Expression



Line shows difference in mean FPKM values between groups.
* Denotes statistical significance ($p < 0.05$)

Conclusions and Implications:

- RNA-seq analysis revealed differential expression in 261 genes, including decreased expression of genes associated with spatial learning and memory in humans and mice.
  - Increased expression of Iron transport genes.
- RRBS analysis identified 346 DMRs and 454 DMSs, the majority of which were found to be hypomethylated in the SID group.
- No global methylation differences were seen between the SID and Control group (data not shown).
- 3 DMRs and 6 DMSs were found to be associated with DEGs (located < 5kb from DEGs).
- Further analysis is required to determine whether DNA methylation is present outside of CpG context in pig brain, as recently reported in human and mouse, and whether these patterns have any effect on gene transcription.

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