Comparative Genomics

Effects of Peripheral Viral Infection on Hippocampal DNA Methylation and Gene Expression Patterns in a Pig Model of Cognitive Development

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Introduction:

- Neonatal environmental insults can affect hippocampal development and function, and infectious disease is the most common cause of illness in children; however, little is known about the long term effects of neonatal peripheral infection on brain development and function.
- 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 Infected, 5 Control) used in this study were collected from a previously published study assessing the effects of Porcine Reproductive and Respiratory Syndrome virus (PRRSv) infection on spatial learning and memory in a neonatal pig model of human infants (Figures 1, 2 & 3; Elmore et al., 2014).
- Piglets were infected with PRRSv at 7 days of age.
- At 14 days of age piglet spatial learning and memory assessment began using a clear plastic plus-shaped maze with visual cues.
  - Infected piglets took longer to locate the reward, had fewer correct choices, and showed increased microglia activation and neuroinflammation in the hippocampus.

Figure 1: Plus-shaped Maze Used in Cognitive Development Experiments

Figure 2: Proportion of Activated Microglia Cells in Hippocampus Samples

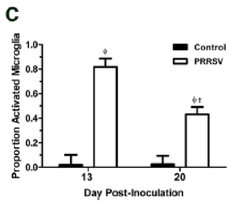
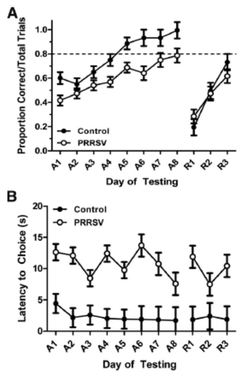
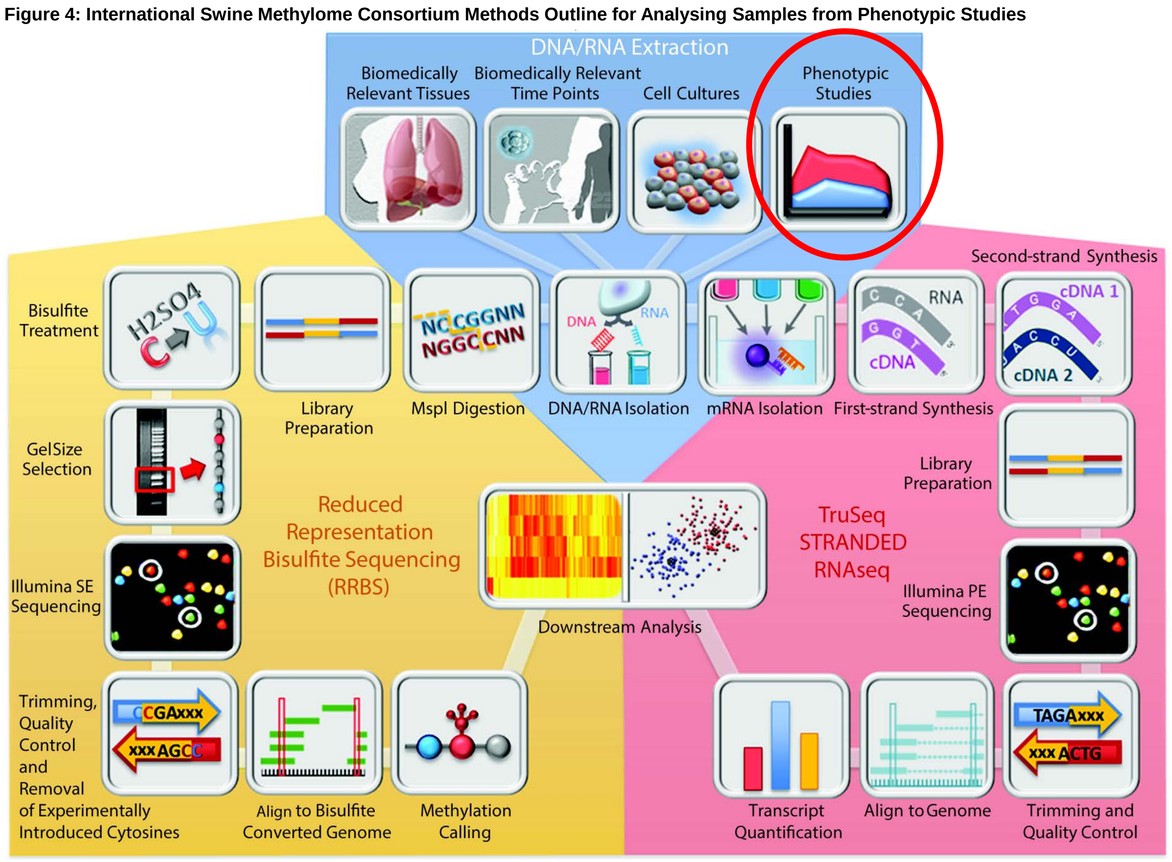


Figure 3: Piglet Performance During the Acquisition Phase (A1-6) and Reversal Phase (R1-3) of Testing for Maze Task



Materials and Methods:

Figure 4: International Swine Methylome Consortium Methods Outline for Analysing Samples from Phenotypic Studies



- Piglets were euthanized at 27 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 performed on all sites with a minimum of 10 reads/site and 25% methylation difference using the R package methylkit (Akalin et al., 2012)
- RNAseq 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
26	Control	31,279,239	58.92%	1.25%	18.63	43.45%	2,163,874	913,441
27b	Control	40,047,008	58.62%	1.40%	23.07	42.75%	2,381,939	1,253,998
30	Control	42,225,575	59.55%	1.39%	23.2	43.72%	2,360,491	1,172,497
34	Control	39,815,876	60.22%	1.30%	20.59	41.57%	2,201,359	768,999
37	Control	38,629,046	58.28%	1.34%	21.51	44.62%	2,283,257	1,038,258
41	Infected	54,630,996	59.76%	1.38%	25.71	42.17%	2,321,900	865,075
45	Infected	62,027,237	59.13%	1.42%	29.41	44.66%	2,365,815	962,044
48	Infected	46,208,224	59.41%	1.38%	25.74	42.09%	2,321,367	954,068
Theoretical Max				1.75%				2,812,047

Figure 5: Average Number of CpG Sites within Genomic Locations

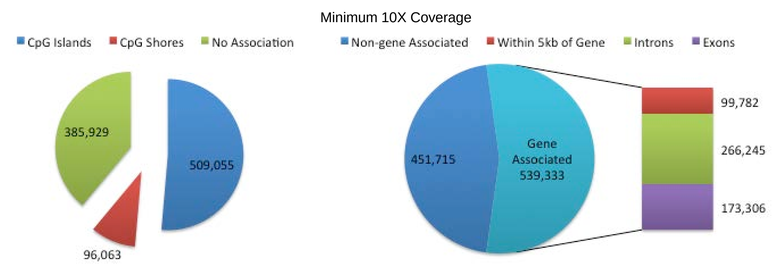


Table 2: Differential Methylation Analysis Between Infected and Control Hippocampus

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
1,224	1,119	451	773	509	610	25	17

Table 3: DEGs Involved in Learning, Memory, and Viral Immune Responses

Gene	Expression	Description
PRSS12	Decreased	Promotes structural reorganizations associated with learning and memory operations. Causes mental retardation when truncated in humans.
RGS14	Decreased	Involved in visual memory processing and hippocampal-based learning and memory.
MEF2C	Increased	Negative regulator of hippocampal-dependent learning and memory.
DHX58	Increased	Involved in innate immune responses to RNA and some DNA viruses.
OAS2	Increased	Antiviral enzyme which recognizes dsRNA and plays a critical role in cellular innate antiviral responses.
IFIT1,2,3	Increased	Antiviral proteins which act as inhibitors of viral processes, including replication and translational initiation.
DDX60	Increased	Positively regulates immune responses to viral infection.
TLR-3	Increased	Mediates innate immune responses to dsRNA associated with viral infections.
IFIH1	Increased	Cytoplasmic receptor for viral nucleic acids.

Figure 6: DEGs Between Infected and Control Hippocampus

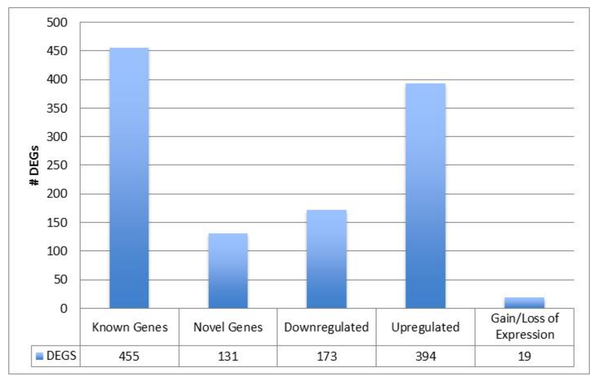
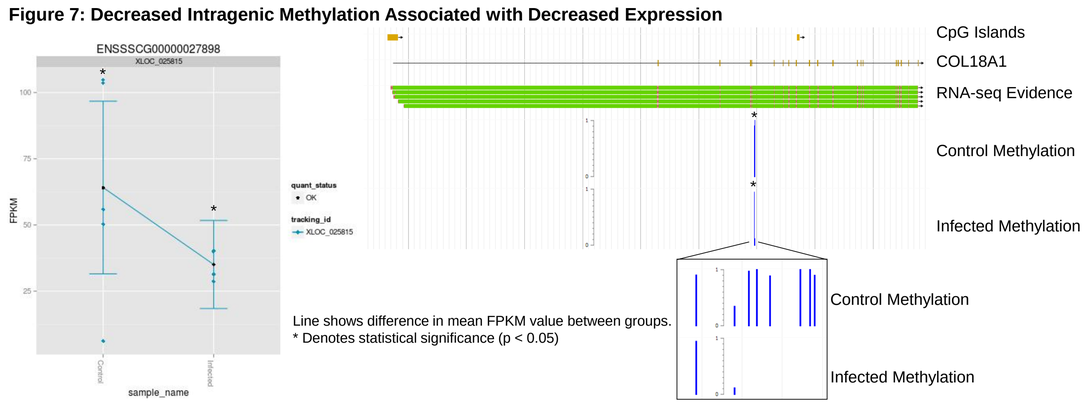


Figure 7: Decreased Intragenic Methylation Associated with Decreased Expression



Conclusions and Implications:

- RNA-seq analysis revealed differential expression in 586 genes, including genes shown to be associated with spatial learning and memory in humans and mice.
  - Increased expression of genes involved in viral recognition and immune responses.
- RRBS analysis identified 1,224 DMRs and 1,119 DMSs, the majority of which were found to be hypermethylated in the Infected group.
- No global methylation differences were seen between the Infected and Control group (data not shown).
- 25 DMRs and 17 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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