Epigenetic Differences of Dairy Cows Classified with Immune Response Traits

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Never start a presentation with a graph...

Never start a presentation with a quiz!!
Identical or Not Identical?
Identical or Not Identical?
Balancing act: Cell senescence, aging related to epigenetic changes

Researchers Discover an Epigenetic Lesion in Hippocampus of Alzheimer’s
Jan. 21, 2014 — New research demonstrates, for the first time, the

Mice Inherit Specific Memories, Because Epigenetics?
by Virginia Hughes

Epigenetics: New Link Between Nutrition, Cancer
Jan. 9, 2014 — In a recent article, researchers explore the possible effects that diet can have on gene expression through epigenetic mechanisms. Explaining the impact of

Grandma’s Experiences Leave a Mark on Your Genes
Your ancestors’ lousy childhoods or excellent adventures might change your personality, bequeathing anxiety or resilience by altering the epigenetic expressions of genes in the brain.
By Dan Hurley I Tuesday, June 11, 2013

Epigenetics of Breast Cancer Family History
Jan. 7, 2014 — Researchers have examined whether a specific epigenetic modification (more specifically, methylation of the DNA) can be associated to breast

Why Your DNA May Not Be Your Destiny
Denise Chow, LiveScience Staff Writer | June 04, 2013 12:52pm ET

Your source for the latest research news
Epigenetics

• “above” genetics – gene and environment interaction
• Acts like a switch - turning genes on and off
• Genomics = the blueprints
• Epigenetics = the engineers

Mechanism of Epigenetics
Epigenetics Role in Animal Health

GENETICS
- SNPs
- EBV

EPIGENETICS
- Histone Modifications
- DNA Methylation

ENVIRONMENT
- Pathogens
- Toxins
- Hormones
- Management

Phenotype
Environmental Influences Over Generations

F1 Generation

F2 Generation

Environmental Factor
The Epigenetic Landscape

↑ DNA Methylation turns OFF genes
↓ DNA Methylation turns ON genes

Biased Immune Responders Cows

Antibody Mediated Immune Response

Parasite

Virus

Bacteria

Fungi

Cell Mediated Immune Response

T-cell

B-cell

AMIR

IL-4

IFN-γ

CMIR

CYTOKINES

Infected Cell

T-HELPER CELLS
The Immune System

AMIR – Extracellular
ex E. coli

CMIR – Intracellular
ex MAP

Generation of Bovine Type 1 and Type 2 Skewed CD4+ T-cells

Harvest:
- Cell culture supernatant for measuring cytokines (ELISA)
- RNA for quantifying transcript (Realtime PCR)
- DNA for methylation profiling (Bisulfite Conversion Assay)
Bisulfite Pyrosequencing

- Magnetic bead isolated T-helper cells
- Extract DNA
- Treat with Bisulfite
- Site of Methylation

PCR Product Capturing

Pyrosequencing
IL-4 and IFNγ Gene Expression after Th1-Th2 Skewing of Isolated CD4+ T-cells (n=5 cows)
DNA Methylation of Bovine Type 1 and Type 2 Skewed CD4+ T-cells

DNA Methylation of IFNG Promoter of Th1-Th2 Skewed Bovine CD4+ T-cells (n=5 cows)

DNA Methylation of IL4 Promoter of Th1-Th2 Skewed Bovine CD4+ T-cells (n=5 cows)
How can we apply this on farm?
Parturition Effects on Immune Response

<table>
<thead>
<tr>
<th>Pregnancy</th>
<th>Parturition</th>
<th>Postpartum</th>
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<tbody>
<tr>
<td>AMIR</td>
<td>CMIR</td>
<td>AMIR/CMIR?</td>
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Peripartum Period

- Peripartum period reflects a period of stress in dairy cows

- Higher occurrences of disease after calving
  - e.g. Mastitis, ketosis, metritis

- Evidence of suboptimal immune response
  - (Mallard et. al., 1998)

- Characteristic shifts in Th1- Th2 responses
  - (Shafer-Weaver et. al., 1999)
High Immune Response™

Cell-Mediated Immune Response (CMIR)

Antibody-Mediated Immune Response (AMIR)
• High and low groups are $\pm 1$ standard deviation from mean
High Immune Response™

Disease Occurrence by Immune Response Category

Methods

Peripartum Period

- 28 days Prepartum
Calving
+4 days Postpartum
+21 days Postpartum

10 H-CMIR/L-AMIR & 11 H-AMIR/L-CMIR cows

Whole Blood as a source of
• CD4+ T lymphocytes (Density gradient and MACS)
• Stimulated with T-cell mitogen (ConA)
• Cell culture supernatant for measuring cytokines (ELISA)
• DNA for methylation profiling (Bisulfite Conversion Assay)

PROC MIXED in SAS9.2.1.; log (concentration) to normalize the data.

Y = μ + GROUP + TIME + CYTOKINE + GROUP*TIME*CYTOKINE + PARITY + ε
GROUP = HiAMIR/LoCMIR or HiCMIR/LoAMIR; Time = -28, +4, +28 Days; Cytokine = IFN, IL4, IL17A; Parity = 1st & 2nd
H-CMIR Produce More Cytokines

H-AMIR Cows  H-CMIR Cows

# p<0.10
H-CMIR Produce More Cytokines

IL-4 Concentration (pg/mL)

-28 Days

+ 4 Days

+ 21 Days

H-AMIR Cows

H-CMIR Cows

* p<0.05
What would we expect to see?

**More** cytokine after stimulation of T-helper cells isolated from H-CMIR

==

**Less** methylation for both cytokines in H-CMIR cows
Bisulfite Pyrosequencing

1. Magnetic bead isolated T-helper cells
2. Extract DNA
3. Treat with Bisulfite
4. PCR Product Capturing
5. Pyrosequencing

Site of Methylation

PCR primer

Sequencing primer

G C T - A G C C T
DNA Methylation of IFNG Promoter from Isolated CD4+ T-cells of H-AMIR (n=11) & H-CMIR (n=10) Dairy Cows
DNA Methylation of IL4 Promoter from Isolated CD4+ T-cells of H-AMIR (n=11) & H-CMIR (n=10) Dairy Cows

% of DNA Methylation

-329 +128 +175 +195

H-AMIR UNSTIM H-AMIR STIM
H-CMIR UNSTIM H-CMIR STIM

*
Conclusion

H-AMIR cows less cytokine production

\[\uparrow\text{DNA methylation}\]

H-CMIR cows more cytokine production

\[\downarrow\text{DNA methylation}\]
What you do today can affect the generations of tomorrow
Thank you

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