The Effects of PRRS Vaccination and WUR Genotype on Blood Gene Expression Response to Co-infection with PRRSV and PCV2 in Pigs

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Porcine Reproductive and Respiratory Syndrome (PRRS) remains a major problem to the global swine industry

- $664 million losses/yr in the U.S. alone (Holtkamp et al. 2013)
- PRRS virus (PRRSV): RNA virus → High mutation rate
- Heterogeneity → Emergence of more virulent strains
Porcine Reproductive and Respiratory Syndrome (PRRS) remains a major problem to the global swine industry

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Possible solutions

- **PRRS vaccine (Vac)**
  - only partially effective

- **Genetic improvement**
  - SNP marker WUR10000125 (WUR)
    - B allele is favorable and dominant to A allele.
Co-infection with PRRSV and porcine circovirus type 2 (PCV2) is commonly observed in field cases

- Both PRRSV and PCV2 can *suppress* the host immune defense system.
- PRRSV can *enhance* replication of PCV2 (Allan et al., 2000).
- PCV2 can *reduce* the efficacy of PRRS modified live virus (MLV) vaccine (Opriessnig et al. 2006).
Co-infection with PRRSV and porcine circovirus type 2 (PCV2) is commonly observed in field cases

- Both PRRSV and PCV2 can suppress the host immune defense system.
- PRRSV can enhance replication of PCV2 (Allan et al., 2000).
- PCV2 infection can reduce the efficacy of PRRS MLV vaccine (Opriessnig et al. 2006).

Effect of PRRS Vac and WUR on Viremia

((Dunkelberger et al. 2017))
Objectives

- To evaluate the effect of PRRS vaccination and WUR genotype on pig blood transcriptome response following the co-infection with PRRSV and PCV2.
- To identify mechanisms involved.
Blood Transcriptome Experimental design

Days Post Vaccination (dpv)

0  4  7  11  14
B  B  B  B

≈3 weeks old

B = Blood Collection for QuantSeq days
Blood Transcriptome Experimental design

- PRRSV infection (KS62) and PRRS MLV strains are heterologous.
• **PRRSV infection (KS62)** and PRRS MLV strains are heterologous.
Methods and Materials for Blood Transcriptome

- **Animal:**
  - 7 pigs for each treatment group:
    - Vac-AA, Vac-AB, nonVac-AA, nonVac-AB

- **Samples:**
  - 191 Blood samples
  - at 4, 7, 11, 14 day post vaccination (dpv, Vac pigs only) and 0, 4, 7, 11, 14, 28 day post infection (dpi)

- **Genome-wide analysis of gene expression:**
  - QuantSeq (3’RNA-seq)

Cost per sample:
- 3’-RNA-seq: < $50
- RNA-seq: ~$400-$500

Slide courtesy of Behnam Abasht
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  - **QuantSeq (3’RNA-seq)**
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    - Filtering globin and genes with low read counts \(\rightarrow\) 5445 genes

Cost per sample:
- 3’-RNA-seq vs. RNA-seq
  - Cost per sample: < $50
  - ~$400-$500

Slide courtesy of Behnam Abasht
Department of Animal Science
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    - QuasiSeq → Differentially Expressed Genes (DEG, q<0.2)
      - Gene expr. = WUR+Vac+WUR*Vac+RIN+Lane

Slide courtesy of Behnam Abasht
Methods and Materials for Blood Transcriptome

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    - Vac-AA, Vac-AB, nonVac-AA, nonVacAB

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      - Gene expr.= WUR+Vac+WUR*Vac+RIN+Lane
    - Ingenuity pathway analysis (IPA) → functional analyses

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Slide courtesy of Behnam Abasht
Department of Animal Science
Results and Discussion: number of DEGs (q<0.2)

- No DEGs for WUR or for WUR × VxStatus.
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- No DEGs for WUR or for WUR × VxStatus.
- For VxStatus, DEGs identified at 4 dpi (n=40) and 7 dpi (n=63).
Results and Discussion: number of DEGs (q<0.2)

- No DEGs for WUR or for WUR × VxStatus.
- For VxStatus, DEGs identified at 4 dpi (n=40) and 7 dpi (n=63).

All 11 overlapping DEGs were less expressed in vaccinated pigs and most related to viral immune response, e.g. MX1, MX2, CXCL10, ISG12(A), CD169, CD64.
 IPA results: Transcriptomic Response in Vac/nonVac

4 dpi

Endocytosis by cells
IPA results: Transcriptomic Response in Vac/nonVac

4 dpi

Endocytosis by cells

Phagocytosis of phagocytes
IPA results: Transcriptomic Response in Vac/nonVac

4 dpi

Endocytosis by cells

Phagocytosis of phagocytes

Immune response of cells
IPA results: Transcriptomic Response in Vac/nonVac

4 dpi

Endocytosis by cells

Phagocytosis of phagocytes

Immune response of cells

Apoptosis
IPA results: Transcriptomic Response in Vac/nonVac

4 dpi
- Endocytosis by cells
- Phagocytosis of phagocytes
- Immune response of cells
- Apoptosis

7 dpi
- Replication of PRRSV
IPA results: Transcriptomic Response in Vac/nonVac

4 dpi
- Endocytosis by cells
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- Apoptosis

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- Replication of PRRSV
- Inflammatory response
IPA results: Transcriptomic Response in Vac/nonVac

4 dpi
- Endocytosis by cells
- Phagocytosis of phagocytes
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- Chemotaxis of phagocytes
- Leukocyte migration
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- Immune response of phagocytes
Conclusions

- The effects of WUR and interactions between WUR and vaccination status were not significant.
  - GBP5 causative gene (Koltes et al., 2015) is not in pig genome build 10.2
Conclusions

- The effects of WUR and interactions between WUR and vaccination status were not significant.

- The effects of PRRS vaccination were significant at 4 dpi (32 dpv) and 7 dpi (35 dpv), which may represent lower innate immune response in vaccinated pigs.
Acknowledgements

USDA-NIFA grant # 2013-68004-20362
Future work

- Pig genome build 10.2 → 11.1 (GBP5)
- +Blood transcriptome assembly data (better 3’ end)
- +Annotation of Iso-Seq data (more isoforms)
Future work

- Pig genome 10.2 → 11.1 version
- +Blood transcriptome assembly data (better 3’ end)
- +Annotation of Iso-Seq data (more isoforms)
- Combine all time points QuantSeq data
- +2nd run QuantSeq
- +QuantSeq with globin block
## DEG (q<0.1) at 4 dpi

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<tr>
<th>Gene stable ID</th>
<th>Gene description</th>
<th>Chrom.</th>
<th>Gene name</th>
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## DEG (q<0.1) at 7 dpi

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</table>
WGCNA results at 4 and 7 dpi

- Module at 4 dpi (cor.=-0.8, p value=2e-06, #genes=166)
  - Four genes are involved in “RIG-I-like receptor signaling pathway” and less expressed in Vac, especially CXCL10, DHX58 as DEG.
WGCNA results at 4 and 7 dpi

- **Module at 4 dpi (cor.=-0.8, p value=2e-06, #genes=166)**
  - Four genes are involved in “RIG-I-like receptor signaling pathway” and less expressed in Vac, especially CXCL10, DHX58 as DEG.

- **Module at 7 dpi (cor.=-0.52, p value=0.01, #genes=105)**
  - In total, nine genes are involved in “cytokine-cytokine receptor interaction”, “chemokine signaling pathway” “NFκB signaling pathway” and “influenza A” and less expressed in Vac, especially DDX58 and MX1 as DEG.
WGCNA results at 4 and 7 dpi

- Module at 4 dpi (cor.=-0.8, p value=2e-06, #genes=166)
  - seven genes are involved in “natural killer cell mediated cytotoxicity” and less expressed in Vac, especially TNFSF10 as DEG.
WGCNA results at 4 and 7 dpi

- Module at 4 dpi (cor.=-0.8, p value=2e-06, #genes=166)
  - seven genes are involved in “natural killer cell mediated cytotoxicity” and less expressed in Vac, especially TNFSF10 as DEG.

- Module at 7 dpi (cor.=0.6, p value=0.003, #genes=229)
  - In total, six genes are involved in apoptosis and less expressed in Vac, especially S100A9 as DEG.
WGCNA results at 4 dpi (32 dpv)

• Module 2 (cor. = 0.64, p value = 7e-04, #genes = 39)
  - the most significantly positively correlated with vaccination status
  - seven genes are involved in “oxidative phosphorylation” and more expressed in Vac, especially ND3, ND4, CYTB as DEG.

The process to form ATP in mitochondria

- ND1, ND3, ND4
- COX7A1
- COX2, COX3
- CYTB
- APT6
DEGs (q<0.2) less expressed in PRRSV vaccinated pigs than non-vaccinated pigs

DEGs (q<0.2) more expressed in PRRSV vaccinated pigs than non-vaccinated pigs

PRRS vaccination led to changes of gene expression that were predicted to decrease immune response of cells and increase viral infection.
IPA results at 4 dpi (32 dpv)

mir-21

DHX58  CXCL10  SIGLEC1  FCGR1A

Immune response of cells  Endocytosis by eukaryotic cells
IPA results at 7 dpi (35 dpv)

PRRS vaccination led to changes of gene expression that were predicted to decrease inflammation response, replication of PRRSV, and immune response of phagocytes.

PRRS vaccine → Genes → Inflammatory response → Immune response of phagocytes → Replication of PRRSV
IPA results at 7 dpi (35 dpv)

DEGs (q<0.2) less expressed in PRRSV vaccinated pigs than non-vaccinated pigs
DEGs (q<0.2) more expressed in PRRSV vaccinated pigs than non-vaccinated pigs

PRRS vaccine decreased expression of genes that activate chemotaxis of phagocytes and that inhibit infection, and may increase quantity of blood cells and decrease leukocyte migration.
Viral load (area under the curve)

- PPRSV viremia: 0-21 dpi
  - A portion of pigs enter a rebound phase after 21 dpi
  - A property of the virus rather than host genetics

- PCV2 viremia: 0-42 dpi
  - Much noisier
  - No clear evidence of rebound
Additional slides for potential questions

- PRRS MLV:
  - a 2-ml dose administered intramuscularly
  - Ingelvac PRRS MLV; Boehringer Ingelheim Animal Health; GenBank accession no. AF159149
- Co-infection on 28 dpv:
  - PRRSV:
    - 2-ml dose of $10^5 \text{ TCID}_{50}$ PRRSV
    - isolate KS62; GenBank accession no. KM035803
  - PCV2:
    - $10^{3.6} \text{ TCID}_{50}$ PCV2b (GenBank accession no. JQ692110)
    - administered intranasally and intramuscularly
Blood QuantSeq Samples

191 samples for FWD QuantSeq

Vaccination status

WUR genotype

Littermates

Time points (day post vaccination)

Number of samples for QuantSeq by days post coinfection and treatment groups

- AA-Vac
- AB-Vac
- AA-nonVac
- AB-nonVac

Number of samples for QuantSeq by days post vaccination

Days post vaccination:
4, 7, 11, 14, 28, 32, 35, 39, 42, 56 dpv
Filtering:
• Removed Globin reads
• Removed reads from genes with average read count <2 across samples
• Removed reads from genes with read counts > 0 for less than 3 samples

⇒ 5,445 genes expressed in blood on average
PCA plots

4 dpi

7 dpi
DEGs between Vaccinated vs. Non-vaccinated

Red = more highly expressed in the vaccinated pigs at time point X

Green = more highly expressed in the non-vaccinated pigs at time point X

upregulated
downregulated
Cell enrichment analyses results

- Cten was used to predict enriched cell types from DEG between Vac and nonVac pigs within 4 and 7 dpi.

The observed differences in gene expression may result from differences in immune cell composition in blood.
WGCNA results at 7 dpi (35 dpv)

- Module 1 (cor.=-0.52, p value=0.01, #genes=105)
  
  - In total, nine genes are involved in “cytokine-cytokine receptor interaction”, “chemokine signaling pathway” “NFκB signaling pathway” and “influenza A” and less expressed in Vac, especially DDX58 and MX1 as DEG.