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The Role of Host Genotypes in the Susceptibility to Porcine Circovirus Associated Diseases

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Introduction

- Porcine Circovirus 2 (PCV2) is a small (1.7kb), circular, single-stranded DNA virus. Infected pigs become seropositive between 6-18 weeks of age with 5-15% eventually displaying clinical signs of Porcine Circovirus Associated Diseases (PCVAD). Vaccination is costly given the small percentage of affected pigs.
- PCVAD is usually expressed between 90-120 days of age and commonly manifests as Post-weaning Multi-systemic Wasting Syndrome (PMWS), Porcine Dermatitis and Nephropathy Syndrome, Porcine Respiratory Disease Complex, enteritis, reproductive failure and enlarged lymph nodes / lymphoid depletion.
- Previous research using genome wide association analysis uncovered two SNPs that explain 12.4% (SSC12) and 3.7% (SSC7) of the genetic variation for viral load, respectively. The position of the SNP located on SSC12 was initially unassigned but was putatively located to the proximal end of SSC12 based on linkage disequilibrium. The *CC* genotype of this QTL was found to be associated with lower viral load ($p<0.0001$) and higher ADG ($p<0.05$) when compared with both the *CT* and *TT* genotypes (Engle et al., 2014).
- SOCS3 is a candidate gene identified through functional analysis of potential protein coding genes located in the QTL region mapped on SSC12.
- SOCS3 is a cytokine signaling suppressor gene involved in the function of the mammalian immune response.

Objectives

- Identify genomic regions, genes and mutations associated with phenotypic variance of pigs infected with PCV2.
- Provide alternative/complementary approach to vaccinations.
- Improve the understanding of the swine immune system.

Background

SSC12 Scaffold Development

Initial annotation of a 1.34 Mb contig of the proximal end of SSC12

- The contig and scaffold were assembled using long-read sequencing.
- GENSCAN software and BLAST were used to predict 13 potential protein coding genes in the QTL region.

19Mb scaffold of the proximal end of SSC12

- Approximately 600 SNPs, previously unmapped or mapped to SSC12, were assigned to the scaffold using BLAT. This included the QTL marker previously putatively assigned to SSC12.

Association analysis using Bayes-IM

- Run for viral load on the scaffold alone as well as the whole genome.

Figure 1. Association analysis for viral load on SSC12 19Mb scaffold. Each dot represents a 50kb window of the scaffold with the x-axis representing position of window on the scaffold and the y-axis representing model frequency.

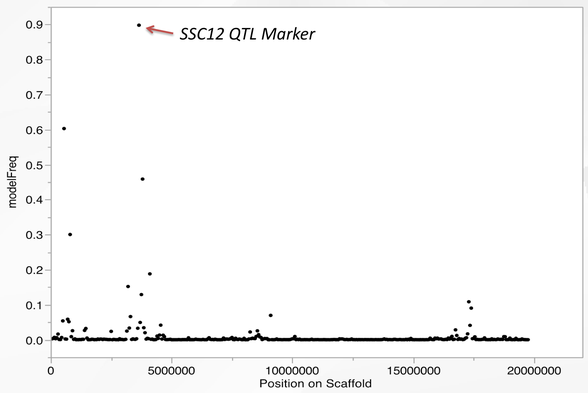
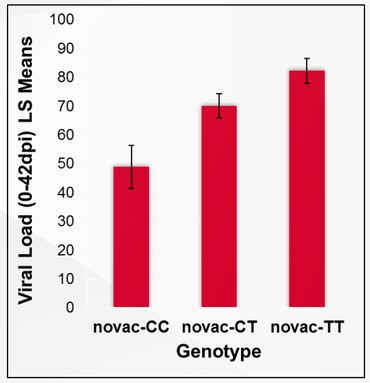


Figure 2. Viral Load (0-42 dpi) least square means (LSM) by genotype for PCV2 infected and non-vaccinated animals.



Findings:

- A Bayes-IM (Interval Mapping) association analysis on the new scaffold confirms the major effect of the QTL genotype on viral load with the CC-genotype being favorable (Figure 1).
- The relationship of the favorable CC-genotype with reduced viral load was further validated with a preliminary experimental infection study on animals selected by genotype (Figure 2).
- Functional annotation of the 13 potential protein coding genes revealed sSOCS3 (suppressor of cytokine signaling 3) as the most likely gene to be associated with the observed variation in susceptibility.

Methods

sSOCS3 Sequencing:

- Tail Clippings- DNA Isolation
- Genotyping via KASP method for QTL marker
- PCR to amplify specific SOCS3 mRNA and promoter region
- Identification of polymorphisms in SOCS3 using Sequencher

SOCS3 Expression:

- Collected serum in Tempus Blood collection tubes at 0, 7,14, 21 and 28 days post-infection and RNA isolation
- cDNA synthesis from isolated RNA
- Measured expression across time points via RT qPCR

Results

Figure 3. SOCS3 expression profile in QTL genotypes following experimental infection with PCV2.

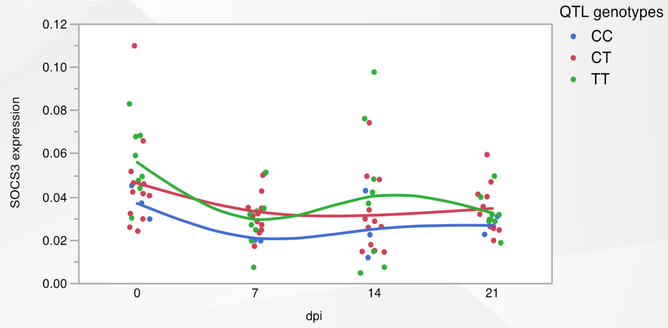


Figure 4. Identified SNPs located in SOCS3 gene and upstream of transcription start site. Blue: SNPs in high linkage disequilibrium with QTL genotypes.

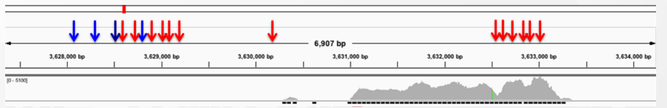
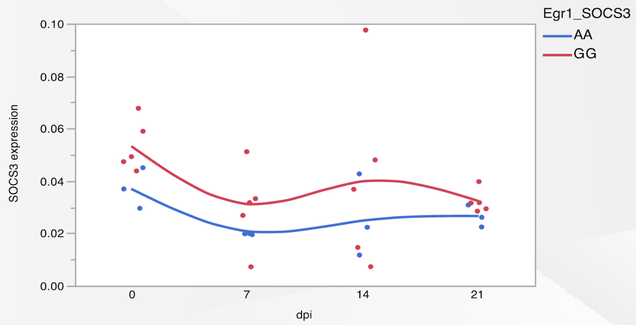


Figure 5. SOCS3 expression profile in pigs with different genotypes of a SNP (-1343 bp) that disrupted an Egr1 motif following experimental infection with PCV2.



Conclusion

- CC QTL genotype is favorably associated with low PCV2 viral load (Figure 3).
- No SNPs associated with the QTL genotypes were found in the coding region of the SOCS3 gene (Figure 4).
- A total of 17 SNPs were found in 3' UTR and upstream of transcription start site (TSS); 4 SNPs located upstream of TSS showed high linkage disequilibrium with the QTL genotypes. A SNP that affected an Egr1 motif showed a suggestive difference in expression between genotypes at 0 dpi ($p= 0.053$, Figure 5).
- Future work will determine if the lower expression of SOCS3 is due to the Egr1 site polymorphism and cause the variation in susceptibility.