# Identifying Molecular Differences in Pigs with Extreme Phenotypes for Weight Gain and Viral Load in Response to PRRS

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### Summary and Implications

Blood transcriptome analyses in the early phase after infection with the Porcine Reproductive and Respiratory Syndrome virus (PRRSv) revealed differential gene expression patterns and regulatory networks between pigs with extreme phenotypes for weight gain and viral load. Understanding these differences could lead to identifying biomarkers that would predict which pigs would be more resistant to PRRS.

### Introduction

In the United States, PRRS is one of the most economically devastating diseases currently in the swine industry. However, the existing variability in pig response to a PRRSv infection, and recent demonstration of significant genetic control of such responses, leads us to believe that selection towards more resistant pigs could be a valid strategy to reduce its economic impact on the swine industry. In the last couple of years, many porcine gene expression studies have been executed in an attempt to unravel the porcine immune responses evoked by the PRRS virus. As part of the PRRS Host Genetics Consortium (PHGC), in this study, a whole blood microarray was conducted contrasting pigs that, although infected, grew as fast as non-infected controls and/or did not show high serum viremia levels with pigs that suffered severely from the PRRSv infection. Two approaches were taken. First, conventional differential expression (DE) analyses looked for significantly differently expressed genes between the extreme groups of animals. Second, we used co-expression methods to identify groups of genes that behave similarly during infection and thus may constitute a coordinated immune response network.

### Materials and Methods

One hundred pigs, with extremely different weight gain and viremia levels after a PRRSv infection, were selected from a total of 600 animals infected with the NVSL 97-7895 PRRSv isolate. A microarray experiment was conducted on whole blood RNA samples taken at 0 and 4 days post infection (dpi) (Figure 1) to measure the expression of the majority of all known genes in blood. Phenotypes examined were weight gain from 0 to 42dpi and viral load from 0 to 21dpi. We also looked at the effect of the WUR10000125 (WUR) genotype on expression levels, which has been associated with a significant portion of the genetic variability in response to PRRS (Boddicker et al., J Anim Sci. 2012, 90(6):1733-46).

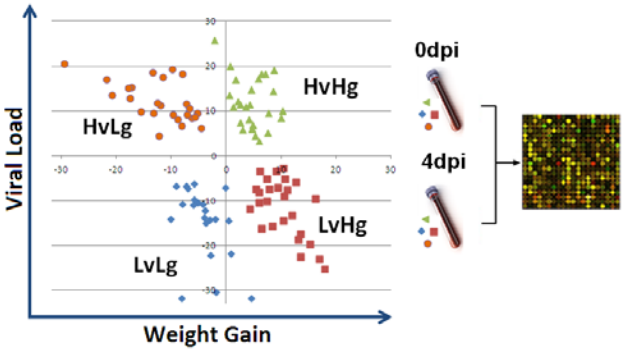


**Figure 1. Animals with extreme phenotypes for viral load (low viral load (Lv) versus high viral load (Hv)) and weight gain (low weight gain (Lg) versus high weight gain (Hg)) were selected to analyze gene expression at 0 and 4dpi using microarray.**

DE analyses were performed to examine the expression profile of extreme phenotypes in the 4dpi-0dpi dataset. Subsequently, Weighted Gene Co-expression Network Analysis (WGCNA) was used to cluster highly correlated genes and to find clusters that were highly informative for

the traits examined. To see if the significant correlated clusters were primarily due to an up- or down regulation of expression of genes in that cluster, or whether gene expression could (partly) be explained by specific cell types, a cell type enrichment (CTEN) analysis was performed. Lastly, Partial Correlation and Information Theory (PCIT) was used to investigate different regulatory networks between extreme phenotypes and animals with a different WUR genotype.

## Results and Discussion

DE analysis between the gene expression levels at 0 and 4dpi showed an up-regulation of several immune response genes, but little or no biological information could be obtained contrasting pigs with growth or viral load extreme phenotypes, nor between animals with a different WUR genotype in the 4dpi-0dpi dataset. Thus, no individual genes that could be tested as a biomarker predicting weight gain or viral load outcomes were found. However, using network based approaches, where the combined expression patterns of sets of genes are used, differences in these extreme groups could be identified. Several interesting clusters of genes were found when applying WGCNA on the 4dpi-0dpi dataset. The overall expression patterns of one such cluster, containing numerous immune response genes such as cytokines, chemokines, interferon type I stimulated genes, apoptotic genes and genes regulating complement activation, was correlated with weight gain (p-value = 0.03) and WUR genotype (p-value = 0.04) across the 100 animals. This cluster was enriched for CD33+ myeloid cells and CD14+ monocytes, important actors in the innate immune system. In addition, PCIT found specific regulatory genes with different connections to their target genes between the phenotypic divergent groups and animals with a different WUR genotype. These regulatory genes could be interesting candidate immune function genes against PRRSv. An analysis of the functions known for these genes revealed that the altered connectivity of these genes was mainly found in adaptive immune pathways.

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