Analysis of Gene Expression in a Region Associated with Host Response to Porcine Reproductive and Respiratory Syndrome Virus Challenge

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Summary and Implications

Previous work identified a 1 Megabasepair region encompassing a quantitative trait locus (QTL) on *Sus scrofa* chromosome 4 associated with response to PRRS virus infection, in terms of weight gain and PRRS viremia. To identify candidate genes in the region responsible for these effects, we evaluated the expression of genes in the region by RNAseq using RNA isolated from whole blood taken from 8 pairs of littermates at 5 time-points following experimental infection with the PRRS virus. Each littermate pair included one individual with the favorable genotype (AB) for the SSC4 region and one with the unfavorable genotype (AA). A comparison of the gene transcript counts between AB and AA individuals was performed with a statistical model that also used sequence information to identify transcripts with allele-specific expression (A versus B) in the AB individuals. These analyses revealed a few candidate genes, with one gene in particular being differentially expressed at multiple time-points and having a high level of overall gene expression. This candidate gene is promising for follow-up functional biology studies to validate and detail its role in host response to PRRS virus infection.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is currently the most economically important disease affecting the United States swine industry. A single-stranded RNA virus causes this disease, which results in reproductive problems in breeding animals and respiratory problems and reduced performance in growing animals. Efforts to produce a vaccine to protect pigs against PRRS virus have generally been unsuccessful due to the unique genetics of the virus and its ability to subvert early innate immune responses. Hence, work is underway to improve the genetics of the host with respect to PRRS virus infection response as an alternative or complement to vaccination.

High-density genotyping microarrays have allowed geneticists to identify single-nucleotide polymorphisms, or SNPs, in the genome that are associated with favorable response to PRRS virus infection. SNPs in a genomic region may co-segregate with high or perfect concordance (linkage disequilibrium), so that they all show association with a linked QTL that controls variation in host response to infection. This result makes identifying a single or small set of causative genes based on genotyping alone difficult. By following up the genotyping work with high-throughput sequencing of RNA transcripts, geneticists can get a more precise understanding of which genes are expressed differently between individuals with favorable versus unfavorable genotypes.

Previous genotyping work by our group identified a QTL within a 1 Megabasepair region on *Sus scrofa* chromosome 4. Individuals with the favorable genotype (AB or BB) showed higher weight gain through 42 days post-infection and lower viremia through 21 days post infection compared to individuals with the unfavorable genotype (AA). The objective of this study was to identify differentially expressed genes in this 1 Megabasepair region based on a gene expression analysis in AB versus AA individuals through RNAseq.

Materials and Methods

Whole blood samples were collected in Tempus tubes from 8 littermate pairs, each with one AB and one AA genotype pig, taken from trial 3 of the PRRS host genetics consortium. The samples were obtained from every pig at each of five time-points: days 0, 4, 7, 10, and 14 post-infection. Sample RNA extraction and sequencing included reduction of globin transcripts, preparation of sequencing libraries, and sequencing using the Illumina HiSeq platform. Bioinformatics processing of the RNAseq data produced a set of predicted RNA transcripts common to all samples and expression counts for each transcript in each sample.

Expression levels were compared between the AB and AA individuals at each time-point using a statistical model of the normalized counts using the nlme package in the R statistical software. Additionally, 136 SNPs were identified in the 1 Megabasepair region for which animals had

genotypes that were consistent with the AB versus AA genotype. For these SNPs, allele specific expression (ASE) was determined using the RNAseq data by analyzing allele counts from the AB individuals in a statistical model to compare the frequency of alternate alleles (associated with B allele) versus reference alleles (associated with the A allele) using the lme4 package in R.

Results and Discussion

A total of 8 known genes are located in the 1 Megabasepair QTL region on chromosome 4, with a total of 12 distinct RNA transcripts. One candidate transcript had a high overall level of expression and showed higher expression in AB individuals at all time-points, except day 0. This difference in expression was statistically significant ($P < 0.05$) at days 7 and 10 post infection. The ASE analysis showed that the SNPs located in this candidate transcript's exonic regions had higher ($P < 0.05$) expression of the B versus the A allele, confirming the greater expression in AB individuals. Other genes showed differential expression at only one time-point and a more varied profile of allele-specific expression. The candidate gene will be a particular focus for follow-up validation, further characterization, and functional studies to determine its biological role in favorable host response to PRRS virus infection.

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