ABSTRACT: Porcine Circovirus 2 (PCV2) is the causative source of a group of associated diseases (PCVAD) that affects production efficiency and can lead to mortality. Using commercial crossbred pigs (n = 974) experimentally infected with PCV2b we analyzed genetic sources of the variation in PCVAD susceptibility. A genome-wide association study including 36,433 SNPs uncovered two major SNPs that explain 11.5% (SSC12) and 2.8% (SSC7) respectively, of the genetic variation for viral load. These SNPs partially explained the negative correlations between viral load and ADG during challenge (r = -0.36, P < 0.0001). The CC genotype of the SNP located on SSC12 was associated with lower viral load (P < 0.0001) and higher overall ADG (P < 0.05) compared to CT and TT genotypes. These genetic variants influence the ability of the host to react and influence PCV2b replication and immune response and improve general animal health and welfare while reducing production costs.

Key words: PCV2; Genetic Susceptibility; Genome-wide association study

Introduction

Porcine Circovirus 2 (PCV2) is the essential pathogen of a group of associated diseases (PCVAD) characterized by weight loss, diarrhea, interstitial pneumonia, nephritis, reproductive failure, dermatitis and lymphoid depletion leading to reduced immunity and susceptibility to other pathogens. PCV2 impairs the immune response, by suppressing the innate immunity role of Natural Interferon Producing Cells (NIPCs) in the maturation of myeloid dendritic cells (McCullough et al. (2009)). Vaccination is effective in controlling PCVAD. A common practice is to vaccinate all pigs, increasing production costs, even though many naturally infected pigs do not express disease.

Variation in susceptibility to PCVAD is influenced by multiple factors including host genetics, exposure to secondary pathogens and management (Madec et al. (2008)). Several reports uncovered breed differences in PCVAD incidence and severity in experimental and natural infections with PCV2 (Zhou et al. (2006); Opiressnig et al. (2006, 2009)). Recent studies quantified the contribution of host genetics in the variation of PCVAD susceptibility in natural (Bates et al. (2009)) and experimental infections (McKnite et al. (2014)) with PCV2. Using an experimental infection approach based on a growing pig model we investigated genetic sources of differences in PCVAD susceptibility and showed that host genetics plays a role in the observed differences in viremia and specific PCV2 immune responses (McKnite et al. (2014)). Herein, we expanded our study by sampling additional maternal lines from several major North-American swine breeding programs in order to help refine QTL locations.

Materials and Methods

Experimental design. Animal use and experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Nebraska - Lincoln (UNL). The experimental challenge was carried out in nine batches (B1 to B9) with 81 to 141 pigs per batch. Barrows were used in B1, B4 and B5-B9 and barrows and gilts were used in B2 and B3. We used maternal and terminal crossbred pigs produced at University of Nebraska (n = 386) and commercial maternal crossbred pigs (n = 588) provided by members of PigGen Canada. Pigs, 3 per litter, from 320 litters by parity 1 to 9 dams were used. Dams had been vaccinated for PCVAD at 3 weeks of age with a single dose of Inglvac CircoFLEX vaccine (Boehringer Ingelheim, St. Joseph, MO). Source farms also included vaccination programs for Porcine parvovirus, Erysipelothrix rhusiopathiae, Clostridium perfringens, Leptospirosis and Colibacillosis and were tested negative for Porcine Reproductive and Respiratory Syndrome Virus (PRRSV). Pigs were colostrum fed and raised under similar conditions. Prior to infection all experimental pigs tested negative for presence of PCV2 and had a sample/positive ratio (S/P) lower than 0.3 for passive IgG and lower than 0.4 for IgM, the specific PCV2 maternal antibodies (McKnite et al. (2014)). These S/P thresholds differentiate pigs that are actively infected or that were previously exposed to PCV2 (Ingenasa). At an average of 36 d each pig was infected with a titer of 10^6.9 50% tissue culture infection dose (TCID50) intranasally and intramuscularly.

Experimental pigs were monitored daily for clinical signs of disease, and weights and blood samples were collected at 0, 7, 14, 21 and 28 days post infection (dpi). Detailed experimental conditions were described in McKnite et al. (2014). The PCV2b strain used was isolated from a pig that displayed symptoms characteristic to
PCVAD infection. The viral genomic DNA from a set of samples randomly selected across batches was amplified using GoTaq Flexi DNA Polymerase (Promega) and sequenced using dye terminators and ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) to validate the genetics of the PCV2b strain recovered from the infected pigs. Similar protocols were used to sequence positional candidate genes in the SLAII region.

Serum analyses. ELISA (Ingenasa) was used to obtain serum profiles of PCV2-specific IgG and IgM antibodies, which were normalized based on positive controls. Viral DNA was extracted from serum using QIAamp DNA Minikit (Qiagen) and viral copy counts were obtained by quantitative real-time PCR using TaqMan Master Mix and ABI 7900 Real Time PCR System (Applied Biosystems). Viral load was calculated based on viremia estimated at each time point using an algorithm that fitted a smooth curve over the 28 d and summed the areas in increments of 0.01 time units (Boddicker et al. (2012)).

DNA isolation and genotyping. DNA was isolated using DNeasy tissue kits (Qiagen) and genotyped using Porcine SNP60K BeadArray (Illumina). A minimum GenCall genotype quality score of 0.40 and a genotyping call rate of 0.80 were used as thresholds for removing low quality DNA samples and SNP assays (Tart et al. (2013)). As a result, 974 samples profiled by 56,433 SNPs were used in the genome-wide association analysis.

Statistical analyses. The proportion of genetic variance for each trait was estimated using the posterior estimates of the high-density SNPs effects using a Bayes B model (Kizilkaya et al. (2010)) including litter, pen and batch as class variables and passive IgG (IgG at d 0) and age at infection as covariates. Bayesian models were based on π set to 0.99 and 40,000 iterations with the first 1,000 samples being discarded as burn-in. Effects sampled at each 40th iteration were used to generate the posterior distribution for the genetic variance explained by each 1 Mb window (Tart et al. (2013)). Genomic prediction values were estimated for each pig based on individual genotypes and SNP effects as described by Fernando and Garrick (2009). Single marker associations were tested using a linear mixed model that included SNP genotype and batch as fixed effects, age at infection and passive IgG as covariates, and litter and pen as random effects.

Results and Discussion

Variation in viral load influenced ADG during challenge. Viremia was the best predictor of decreased ADG following infection; negative moderate phenotypic correlations between residuals for viremia and ADG were estimated starting with viremia at 14 d post infection (dpi) and ADG during the last two weeks of challenge (r = -0.31 to -0.39; P < 0.001). The pair-wise correlation between ADG (0 - 28 d) and viral load was -0.36, while the correlation between ADG and PCV2 specific antibodies, IgM (-0.12 to 0.05) and IgG (-0.02 to 0.11) were weak. Our previous work showed no association between ADG prior to challenge and viremia during the first two weeks (r = 0.01 to 0.03, P=0.63) or viral load (r=0.02, P=0.73) (McKnight et al. (2014)). The viral genomic DNA sequences of PCV2 isolated from random samples across batches were identical with the DNA sequence of the PCV2b strain used for infection.

Two major loci influenced variation in PCV2b viremia and viral load. The contribution of SNP genotypes to the phenotypic variation of viremia and antibody levels was limited early in the infection but increased after the surge in viral replication and immune response. The proportion of the phenotypic variation explained by SNP variants in weekly viremia varied from 19% (7 dpi) to 52% (14 dpi) and 64% for viral load (Table 1). The influence of SNP genotypes to the variation in immune response varied from 14% (7 dpi) to 60% (14 dpi) for IgM and from 3% (7 dpi) to 44% (21 dpi) for IgG. The proportion of the variation in ADG explained by SNP variants was limited (7% for week 3 and 4 to 13% for week 1 and 16% for overall ADG). Genome-wide association analyses uncovered two major SNPs that explain 11.5% (UNLPCV2.2009) and 2.8% (UNLPCV2.2010) respectively, of the genetic variation for viral load (Figure 1). The position of UNLPCV2.2009 is unknown but was predicted to be located at the proximal end of SSC12 based on linkage disequilibrium estimates (r² > 0.32). The position of SNP UNLPCV2.2010 is located in the vicinity of Swine Leukocyte Antigen II (SLA II), a region known to have a role in immune response. Sequencing analysis of the SLAII region in a selected group of samples detected haplotype and SNP allelic diversity that were in agreement with previous characterizations of the variations at the SLA II locus (Ho et al. (2010), https://www.ebi.ac.uk/ipd/mhc/sla). For example, out of 12 haplotypes present based on the sequences of DQBI alone, haplotype 701 was the most common (25.9%). The diversity is similar with the variation of DRBI reported in outbred populations by Ho et al. (2010). Recent analysis of the influence of host genetics on the immune response in pigs experimentally infected with PRRSV discovered a QTL for antibody level in the same region of SSC7 (Dekkers et al. (2013), personal communication).

Table 1. Proportion of phenotypic variance explained by 56,433 SNPs

<table>
<thead>
<tr>
<th>Trait/dpi</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>0-28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viremia</td>
<td>0.19</td>
<td>0.52</td>
<td>0.45</td>
<td>0.39</td>
<td>0.64</td>
</tr>
<tr>
<td>IgM</td>
<td>0.14</td>
<td>0.60</td>
<td>0.44</td>
<td>0.52</td>
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</tr>
<tr>
<td>IgG</td>
<td>0.03</td>
<td>0.08</td>
<td>0.44</td>
<td>0.38</td>
<td></td>
</tr>
</tbody>
</table>

0-7  7-14  14-21  21-28  0-28

ADG  0.13  0.11  0.07  0.07  0.16

a.
These two polymorphisms explained most of the genetic variance explained by their respective 1 Mb windows (> 94%). The window that potentially harbors SNP UNLPCV2.2009 at the proximal end of SSC12 explained 10.5% of the additive genetic variation for viral load, significantly greater than zero and also greater than the average genetic variance explained by the 1 Mb windows (Prob > 0.99). The second ranked window was located on SSC7 and explained 7.7% of the additive genetic variation for viral load, which was suggestively greater than zero and greater than the average genetic variance explained by 1 Mb windows (Prob > 0.90). Potential genes for the QTL regions include members of the SLA II gene complex for the QTL mapped on SSC7; re-sequencing and re-assembly are necessary to identify potential candidates for the QTL mapped on SSC12.

Figure 2. Genome-wide association analysis between 56,433 SNPs and PCV2b viral load. Each dot represents the proportion of genetic variance explained by each SNP. The X-axis represents the SNP position in the swine genome. The Y-axis represents the individual SNP contribution to the genetic variance for viral load. Alternate colors represent autosomes, from SSC1 to 18, followed by chromosome X and a group of SNPs without a known location.

Figure 1. Variation in PCV2 viremia (a), PCV2b-specific antibody response IgM and IgG (b) and ADG (c) following experimental challenge with a PCV2b strain.
0.68 across the genetic lines used in the study. In general, the direction of the genotype effects of this SNP was consistent across the genetic lines.

**Conclusions**

Genome-wide association analyses uncovered host genetic variation in viremia, immune response and growth during experimental challenge with PCV2b. Individuals that carried favorable alleles from the SNPs located in the SLA II region of SSC7 and in the proximal region of SSC12 had lower viral load and weekly viremia, higher overall and weekly weight gain. These genetic variants are associated with the ability of the host to inhibit PCV2b replication and affect the immune response limiting the influence of PCV2 on general animal health.

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**Literature cited**