Genome-wide association analysis for porcine reproductive and respiratory syndrome virus susceptibility traits in two genetic populations of pigs¹

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ABSTRACT: Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically important pathogen that continues to threaten swine industry sustainability. The complexity and high genetic diversity of PRRSV has prevented vaccines from conferring adequate protection against disease outbreaks. Genome-wide association analyses of PRRSV experimentally infected pigs representing two genetic lines ($n = 174$ to 176) revealed two major genomic regions accounting for ~1.2% of the genetic variation in PRRSV-specific antibody level in serum or lung. The major region for serum antibody was mapped to SSC7 near the SLAI complex, which has also been implicated in susceptibility to other swine

viral pathogens. Haplotype substitution analysis uncovered potential *DQB1* haplotypes associated with divergent effects. A novel major region for lung antibody was mapped to the proximal end of SSC17 with the top SNP overlapping two genes, *PRAG1* and *LONRF1*. Sequencing *LONRF1* uncovered polymorphisms within the coding region that may play a role in regulating PRRSV-specific antibody production in lung tissue following PRRSV infection. These data implicate novel host genomic regions (SSC17) that influence PRRSV-specific immune response as well as a common region (SSC7) potentially involved in susceptibility to multiple viral pathogens.

Key Words: host genetics, immune response, porcine reproductive and respiratory syndrome, susceptibility

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J. Anim. Sci. 2019.97:3253–3261

doi: 10.1093/jas/skz184

INTRODUCTION

Porcine reproductive and respiratory syndrome virus (PRRSV) poses an ongoing threat to the economic sustainability of the swine industry, costing U.S. herds well over \$650 million annually (Holtkamp et al., 2013). Affected pigs exhibit interstitial pneumonia, slow growth, and

in breeding herds, reproductive failure including higher incidence of abortions, mummies, and stillbirths (Lunney et al., 2010). The complexity and high mutation rate of this RNA virus have hindered the development of an effective vaccine necessary for adequate outbreak prevention (Sun et al., 2018). Comparison between different genetic lines experimentally infected with PRRSV provided initial evidence of a genetic component involved in the associated immune response (Petry et al., 2005). In this study, we explored a highly characterized data set of pigs experimentally infected with PRRSV (Petry et al., 2005). The pigs represent two distinct swine populations, Nebraska Index Line (NEI) selected for increased litter size and commercial Hampshire × Duroc

¹This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2016-09374 from the USDA National Institute of Food and Agriculture and by an Animal Health Grant from the Institute of Agriculture and Natural Resources of the University of Nebraska – Lincoln.

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Received December 31, 2018.

Accepted May 30, 2019.

(HD) cross. The HD pigs exhibited elevated body temperature, reduced growth, and increased viremia and viral titers compared with NEI pigs as well as a greater mean pneumonia score, indicating that NEI is less susceptible to PRRSV than the HD genetic line (Petry et al., 2005).

Boddicker et al. (2012) conducted a genome-wide association study (GWAS) on cross-breed barrows ($n = 565$) experimentally challenged to assess the genetic basis of variation in host susceptibility to PRRSV. A region was identified on chromosome 4 (SSC4) associated with PRRSV viral load and weight gain, accounting for 15.7% and 11.2% of the genetic variances, respectively (Boddicker et al., 2012). The top marker within this region, rs80800372 (*WUR10000125*), accounted for >99% of the window variation and had significant allelic substitution effect ($P < 0.0001$) with the *AA* genotype associated with higher viremia and reduced growth compared to the *GG* genotype (Boddicker et al., 2012). In a later study, an intronic SNP (rs340943904) that introduced a splice acceptor site leading to a shift in the reading frame and an early stop codon in *GBP5* was suggested as the causal polymorphism of the QTL discovered on SSC4 (Koltes et al., 2015). Another GWAS, focused on antibody response and reproductive traits in a sow herd naturally infected with PRRSV, revealed two regions on SSC7 that together explained 40% of the genetic variation for PRRSV antibody level (Serão et al., 2014). One of these regions was a 7 Mb window encompassing the Swine Leukocyte Antigen Complex Class II (SLAI) locus, which alone accounted for 25% of the genetic variation. The top SNP, rs80947467 (*ASGA0032151*), located within this window explained ~5% of the genetic variance in PRRSV antibody response (Serão et al., 2014). This region was also identified in an experimental challenge with two PRRSV isolates (Hess et al., 2018). Specifically, the 25 Mb window on SSC7 was associated with PRRSV-specific antibody levels in serum at 42 days post infection for both isolates and accounted for nearly 30% of the genetic variance in the combined analysis (Hess et al., 2018).

To further interrogate the genetic factors influencing the variation in PRRSV susceptibility in the original data set reported by Petry et al. (2005), all of the pigs from the NEI and HD genetic lines were genotyped with the Illumina Porcine SNP60 BeadArray and included in a GWAS for PRRSV viremia, antibody level, and ADG. The ultimate objective of this research is to not only identify genomic regions associated with PRRSV susceptibility but also common regions associated with susceptibility to multiple viral pathogens.

MATERIALS AND METHODS

Population, Experimental Infection, and Sample Collection

The data used in this research were obtained from a previous study that conducted an experimental challenge with PRRSV (Petry et al., 2005). The pigs subjected to a PRRSV challenge represented two genetic lines: the NEI ($n = 100$), a Large White-Landrace composite line selected across 20 generations for increased litter size, and an HD commercial cross ($n = 100$) selected for lean growth (Petry et al., 2005). The NEI pigs were generated at the University of Nebraska swine research facility, while the HD pigs originated from commercial lines. Neither farm had prior exposure to PRRSV; however, each individual was screened for the presence of PRRSV by PCR and PRRSV-specific antibodies using an ELISA test before initiating the challenge. To adequately represent each population, two pigs of the same sex/litter from as many litters as possible were randomly selected resulting in a total of 83 sires and 163 dams represented in this study (Petry et al., 2005).

Littermates were randomly assigned to one of two separate environmentally controlled rooms at the University of Nebraska Veterinary and Biomedical Sciences Animal Research Facility and housed in pens of 12 to 13 pigs of the same genetic line. One room was randomly selected for experimental infection (Petry et al., 2005). Two replicates of 50 pigs per genetic line were completed at the same time across two seasons, resulting in a total of 200 infected pigs (Petry et al., 2005). At an average age of 28 d (NEI) and 23.7 d (HD), selected pigs were experimentally infected intranasally with a 2-mL dose of 10^5 cell culture infectious dose 50%/mL (CCID₅₀) of PRRSV Strain 97-7985 (Petry et al., 2005). Immediately prior to inoculation (day 0) and at 4, 7, and 14 d following inoculation, body temperature by rectal probe, BW, and blood were collected from each individual. Lung, bronchial lymph node (BLN), and spleen samples were collected at necropsy on day 14 and stored at -80°C (Petry et al., 2005).

This study was approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee (Project ID: 1657).

Trait Measurement

Viral infectivity titration on MARC 145 cell cultures was used to measure serum viremia (CCID₅₀/mL) and viral titer in BLNs and lungs (CCID₅₀/g),

as documented in [Petry et al. \(2005\)](#). Overall viral load for each animal was expressed as area under the curve calculated using an algorithm accounting for viremia at each time point following infection (0, 4, 7, and 14 d) that fits a smooth curve and sums the areas split up by 0.01 increments of time ([Boddicker et al., 2012](#)). An ELISA test was used to calculate the level of PRRSV-specific antibody in serum collected 14 d postinfection (dpi), expressed as sample-to-positive ratio with values >0.40 considered positive for PRRSV-specific antibody ([Petry et al., 2005](#)). The presence of pneumonia was expressed as binary scores for each lung sample. In addition, lung sections were also scored subjectively between 1 and 3 by light microscopy for lesions and severity of pneumonia ([Petry et al., 2005](#)).

Genome-Wide Association Analyses

Genomic DNA was extracted from spleen samples using the DNeasy kit (Qiagen). Each of the infected pigs ($n = 174$ to 189) was genotyped with the Illumina Porcine SNP60 BeadArray. A GenCall quality score of 0.40 and genotyping call rate of 80% were used as genotype quality thresholds, resulting in 57,390 SNPs mapped to the *Sscrofa 11.1* assembly. A GWAS was employed via GENSEL software for PRRSV viremia, viral load, antibody level, and ADG using a BayesB model ([Fernando and Garrick, 2008](#)). Pen and a contemporary group (CG) variable comprised of genetic line, replicate, and sex were included as class variables and age at infection was included as a covariate. The Bayesian analysis was based on a 1% probability of a SNP having a non-zero effect ($\pi = 0.99$) and the Markov Chain Monte-Carlo included 41,000 iterations with the first 1,000 discarded as burn-in. The posterior distribution for genetic variance explained by each 1 Mb window was derived from the average of effects sampled every 40th iteration, calculated as the variation of genomic values across animals generated by the SNP effects sampled within the window and presented as a proportion of genetic variance for the entire genome ([Tart et al., 2013](#)). The probability of each window having an effect >0 was estimated considering the null hypothesis that a 1-Mb fragment was not harboring a QTL. Also, the probability of each window explaining a variance greater than the average proportion of variance was estimated under the assumption that the genetic determinism of the targeted traits is infinitesimal and every window accounted for the same proportion of the genetic variation.

Association between phenotypic traits and major DNA markers, selected based on this research and previous studies ([Boddicker et al., 2012](#); [Serão et al., 2014](#); [Walker et al., 2018](#)), was assessed using a linear mixed model fit by JMP Pro 13.1 (SAS Inst. Inc.). The CG and genotype were included as fixed effects, pen nested within CG was included as a random variable and age at infection was treated as a covariate. The estimated additive effect and allelic frequencies were used to estimate the proportion of phenotypic variation explained by each polymorphism. Differences between least square means (LSM) estimated for each genotype were determined by Tukey test.

SLAII Haplotype Assignment and Substitution Effects

SLAII haplotype assignment was based on specific regions of the *DQB1* gene, which is a member of the *SLAII* complex. These regions of *DQB1* were amplified using GoTaq Flexi DNA polymerase (Promega) and sequenced via dideoxy sequencing. The obtained *DQB1* sequences were compared with reference haplotype sequences (Immuno Polymorphism Database, www.ebi.ac.uk/ipd/mhc) generating *DQB1*-based *SLAII* haplotype assignments for each animal. The potential effects of the *SLAII* haplotypes on the phenotypic variation in serum antibody (Ab) titer (14 dpi) were estimated as haplotype substitution effects. Contrasts between haplotypes were estimated using a linear mixed model as described above including one variable for each haplotype with values 0, 1, and 2 corresponding to the animal having 0, 1, or 2 copies of the assigned haplotypes. The haplotype substitution effects were presented as deviations from the mean of the *DQB1*-based *SLAII* haplotypes.

Evaluation of *LONRF1*, a Candidate Gene for PRRSV-Specific Antibody Titer in the Lung

RNA was extracted from spleen samples using the RNeasy kit (Qiagen), and cDNA was synthesized using First Strand cDNA synthesis kit (GE Healthcare). The full-length mRNA sequence of the positional candidate gene for PRRSV-specific antibody titer in the lung, Lon peptidase N-terminal domain and ring finger 1 (*LONRF1*), was generated using LongAmp DNA Polymerase (New England Biolabs), and sequenced via dideoxy sequencing. Sequence comparison between samples using Squencher software was utilized to uncover

potential functional polymorphisms within the coding region. Genotyping *LONRF1* candidate SNPs was performed using KASPar assays (LGC Group). The effect of these SNPs was estimated using a linear mixed model as described above. Differences in lung Ab titer between LSM estimated for each genotype were determined by Tukey test.

RESULTS AND DISCUSSION

Genome-Wide Association Study

The combined effect of 57,390 SNPs accounted for differences in PRRSV susceptibility related traits, explaining 25.2% to 41.8% of the phenotypic variation (Table 1). Despite a substantial proportion of phenotypic variance in viral load (41.8%) explained by the SNP genotypes, no single genomic region accounted for a predominant proportion of the variation. The top 1 Mb windows were located on SSC13 (200 to 201 Mb) and SSC16 (69 to 70 Mb) and together accounted for only 0.33% of the genetic variation (Supplementary Table S1). The probability of these windows having an effect greater than the average window effect was low ($Pr = 0.14$). Only two of the ten top SNPs were located within either of these windows, rs81442504 (*H3GA0037974*, seventh top SNP) on SSC13 (200 Mb) and rs81461846 (*ALGA0091555*, ninth top SNP) on SSC16 (69 Mb).

For PRRSV-specific antibody (Ab) level in serum, a prominent QTL was observed with four out of the six top 1 Mb windows located on SSC7 (Supplementary Table S2). The two top windows were located from 25 to 27 Mb and accounted for 1.22% of the genetic variance ($Pr = 0.19$), highlighting this as the primary QTL region for PRRSV-related immune response (Supplementary Table S2). The SNP with the largest association for serum antibody, rs80857355 (*DRGA0007416*) was located within this SSC7 QTL region at about 26.1 Mb (Figure 1). In the lung, the top window

for Ab titer was located on the proximal end of SSC17 (0 to 1 Mb) and accounted for 1.22% of the genetic variation ($Pr = 0.23$) (Supplementary Table S3), including *ASGA0106200* (769 kb) and rs335100185 (*DIAS0004299*, 885 kb) as top SNP. In the BLN, the top window for Ab titer that accounted for 0.42% of the genetic variation was located on SSC14 (127 to 128 Mb, $Pr = 0.22$) (Supplementary Table S4), with the top SNP represented by rs80881916 (*ALGA0082173*, 127.8 Mb). Interestingly, the fourth top window accounting for 0.21% of the genetic variation in BLN Ab titer is the same window on SSC16 (69 to 70 Mb) that was identified for PRRSV viral load. However, the top SNPs within this region are not common between the two traits.

The top windows for ADG were located on SSC5 (64 to 65 Mb) and SSC11 (74 to 75 Mb) and accounted for 0.73% of the genetic variation ($Pr = 0.20$; Supplementary Table S5). The top SNP, *ASGA0092359*, was located within the SSC5 window (64.8 Mb), while the second and third top SNPs, rs80806301 (*ASGA0052052*, 74.8 Mb) and rs80826642 (*H3GA0032639*, 74.7 Mb), were located on SSC11.

Swine Leukocyte Antigen Complex Class II

The SSC7 QTL associated with PRRSV antibody is located near the Swine Leukocyte Antigen Complex Class II (*SLAII*) locus, which encodes proteins expressed on the surface of professional antigen-presenting cells, such as macrophages and dendritic cells. This locus is both polygenic and highly polymorphic, creating high levels of heterogeneity amongst SLAII proteins, which is necessary to recognize a variety of pathogens. The QTL (25 to 27 Mb) associated with serum Ab is located within the same 7 Mb window previously identified for PRRSV-specific antibody by Serao et al. (2014), corroborating the influence of this region on PRRSV-related immune response. However, the top SNP in our study (*rs80857355*) was not

Table 1 Summary of genome-wide association study for porcine reproductive and respiratory syndrome virus susceptibility traits

Trait	<i>n</i>	Genetic variance	Residual variance	Total variance	Phenotypic variance explained by SNP, %
Viral load	166	13.6	19.0	32.6	41.8
Ab (serum)	167	0.12	0.34	0.46	25.2
Ab (lung)	165	0.37	0.56	0.93	40.0
Ab (BLN)	164	0.31	0.57	0.88	35.5
ADG	168	0.01	0.02	0.03	36.6

BLN = bronchial lymph node.

Figure 1 Genome-wide association analysis of PRRSV-specific antibody level in serum using BayesB. Each dot represents an individual DNA marker with the genomic position represented by the x -axis and the proportion of genetic variance represented by the y -axis. Each chromosome (1 to 18) is represented by a different color with the last group designating all unmapped markers.

the same as the top SNP in the [Serao et al. \(2014\)](#) data set (*rs80947467/ASGA0032151*, 26 Mb). Interestingly, this QTL was also located adjacent to a QTL on SSC7 (24 to 25 Mb) identified for PCV2 viral load and antibody level following experimental infection with a PCV2b strain ([Walker et al., 2018](#)). The top SNP within this QTL for PCV2 viral load was *rs80874530 (ALGA0039682)* and *rs80794785 (ALGA0039710)* located at about 24.5 and 24.8 Mb, respectively ([Walker et al., 2018](#)). Although the QTL do not directly overlap, the close proximity to *SLAII* suggests a potential role of this locus in both PRRSV and PCV2 susceptibility. The level of serum PRRSV Ab was higher in HD compared with NEI, a negative relationship being reported for this trait with viremia ($r = -0.02$ to -0.11 ; [Petry et al., 2005](#)).

SLAII haplotypes were assigned to individual pigs (NEI = 72, HD = 77) by sequencing the highly polymorphic regions of the *DQB1* gene. There were seven classical haplotypes identified in NEI and nine haplotypes identified in HD. *DQB1 0203* was the major haplotype in NEI (67.4%), with the homozygote for this haplotype representing the predominant genotype at this locus (50.0%). In HD, *DQB1 0201* was the major observed haplotype (43.1%), with the homozygote for this haplotype representing the predominant genotype (33.8%). Heterozygosity level at this locus was larger in HD (44.2%) compared with NEI (33.3%). The

predominant heterozygotes were *0101/0203* in NEI (16.7%) and *0201/040201* (23.3%) in HD.

Haplotype substitution analysis demonstrated that *DQB1*-based *SLAII* haplotypes have significant effects on serum PRRSV Ab ($P < 0.01$). The two major haplotypes identified, *DQB1 0201* (25.2% overall frequency) and *DQB1 0203* (31.8%), were associated with the largest and divergent haplotype effects. Haplotype *DQB1 0201* was associated with a decrease while *DQB1 0203* was associated with an increase in serum Ab compared with the average haplotype effects ($P < 0.005$). Additional effects were observed for two minor haplotypes, *DQB1 0101* (8.0%) and *DQB1 040201* (3.1%; $P < 0.05$).

Taking into account the multi-breed genetic composition of the lines, we hypothesized that the QTL associated with PRRSV susceptibility are polymorphic in both lines. Therefore, any observed effects are a result of differences in allelic frequency between lines at the QTL. Interestingly, the two major haplotypes identified are either line specific or predominantly present in one of the lines. For example, *DQB1 0203* is only present in NEI and the predominant haplotype in this line (67.4%), while *DQB1 0201* is the major haplotype in HD (43.1%) with a limited presence in NEI (5.2%). We hypothesized that the observed haplotype effects originated within line since the role of population structure was eliminated by including the genetic line in the statistical model.

While the *SLAII* locus is large and the effect on PRRSV Ab could involve multiple genes and genetic variants, at the *DQB1* gene level the major effects associated with Ab were observed for *DQB1 0201* and *DQB1 0203* haplotypes. The difference between these haplotypes at the protein level involves eight residues, including four conservative (*DQB1 p.Leu18Val*, *DQB1 p.Phe50Tyr*, *DQB1 p.His69Tyr*, and *DQB1 p.Asn214Ser*) and nonconservative (*DQB1 p.Phe45Gly*, *DQB1 p.Arg87Leu*, *DQB1 p.Lys103Met*, and *DQB1 p.Thr109Arg*) substitutions. All nonconservative substitution are part of the antigen recognition domain including components of peptide-binding pockets, such as *DQB1 p.Phe45Gly* and *DQB1 p.Lys103Met*. Amino acid profiles at MHC/SLAII locus can cause structural changes in peptide-binding pockets that may influence peptide binding, selectivity, and presentation (Menconi et al., 2008). Further detailed analysis of this locus will be conducted to assess the role of the entire SLAII locus in susceptibility to PRRSV and other viral pathogens

SNP Effects Across Traits

The effects of eight major SNPs identified in this study or previous related research (Table 2; Boddicker et al., 2012; Serão et al., 2014; Walker et al., 2018) were evaluated across the traits measured in this study using a single marker association analysis. The only significant relationships between the genotypes and the selected traits were additive with no dominance effects being detected. The largest effect was observed for *rs80857355* genotypes, explaining 12.6% of the phenotypic variation in the level of PRRSV Ab in serum ($P < 0.0001$). The *AA* genotype associated with high Ab was the predominant genotype in NEI (71.8%) compared with HD (10.1%). A major SNP (*rs80947467*) associated with serum Ab in Boddicker et al. (2012) explained 9.5% of the phenotypic variation ($P < 0.0001$). Similarly, the *TT* genotype (*rs80947467*) associated with high Ab was the predominant genotype in NEI (70.1%) compared with HD (9.0%). The effects of these two SNPs were specific to PRRSV Ab, no relationship with other traits being detected ($P > 0.22$). Similarly, the major SNP located in the QTL region for lung Ab (SSC17), *ASGA0106200*, explained 7.5% of the phenotypic variation ($P < 0.0001$) and had no effects on any other traits ($P > 0.07$).

Two of the SNPs identified in the literature to have effects on disease susceptibility either to PRRSV (*rs80800372*; Boddicker et al., 2012) and PCV2 (*rs80874530*; Walker et al., 2018) were

associated with multiple traits, including PRRSV viral load, lung, and BLN Ab titer. In our data set, the direction of effect for *rs80800372* was the same as in Boddicker et al. (2012) with the *AA* genotype associated with higher viral load compared with the *AG* or *GG* genotype ($P < 0.01$; Table 2). Interestingly, no major QTL for viral load was observed on SSC4 in our study despite a frequency of 28.3% for the *G* allele. However, this could be a result of data less than half the size of that in Boddicker et al. (2012) and the fact that the rare *GG* genotype (9%) was only present in NEI. Interestingly, the direction of the effect for *rs80800372* was the same in both lung and BLN and corresponded with the direction identified for viral load, with the *AA* genotype exhibiting higher levels of PRRSV antibody and viral load than the *AG* or *GG* genotypes (Table 2). This similarity in effect may indicate that the antibody in the lung and BLN is associated or even dependent upon the amount of virus during the infection. A positive relationship like this is to be expected since generally the more virus present the more antibodies produced (Engle et al., 2014). However, this trend is most evident later in the infection (Engle et al., 2014), which makes sense biologically since adaptive immunity responsible for pathogen-specific antibodies (i.e., IgG) is not activated immediately upon infection.

LONRF1 Candidate Gene for PRRSV-Specific Antibody Titer in the Lung

The putative novel QTL on SSC17 (0 to 1 Mb) alone accounted for 1.22% of the phenotypic variation in lung Ab titer (14 dpi). Two human homologues were identified in this region, *PRAG1* (749,508 to 793,345 bp) and *LONRF1* (884,096 to 917,138 bp). The top two SNPs with significant effects on lung Ab titer overlapped the *PRAG1* (*ASGA0106200*, 769 kb) and *LONRF1* (*rs335100185*, 885 kb) genes.

LONRF1 is a protein-coding gene that is expressed in a variety of tissues, but is highly expressed in alveolar macrophages and blood cells (www.BioGPS.org). Gene ontology of *LONRF1* includes the antigen processing, ubiquitination and proteasome degradation pathway, specifically in reference to Class I MHC-mediated processing and presentation (Reactome). This gene has also been identified as a component of dendritic cell maturation in response to various immunostimuli, in which functional remodeling of the ubiquitin-proteasome system occurs (Ebstein et al., 2009). For example, *LONRF1* was upregulated approximately

Table 2 Additive (P^1) and overall (P^2) effects of selected major SNP on porcine reproductive and respiratory syndrome virus susceptibility traits

SNP	Chr	Position, bp	Alleles	Trait	P^1	P^2	LSM ($\pm$ SE)			Proportion of phenotypic variation explained, %
							11	12	22	
rs80800372 (Boddicker et al., 2012)	4	127,441,677	A/G	Viral load	0.0181	0.0607	48.98 (± 0.682)	47.26 (± 0.762)	45.88 (± 1.474)	2.13
				Ab (serum)	0.8353	0.8370	1.237 (± 0.060)	1.179 (± 0.072)	1.259 (± 0.171)	0.03
				Ab (lung)	0.0386	0.1109	4.357 (± 0.096)	4.158 (± 0.111)	3.824 (± 0.241)	2.51
rs80874530 (Walker et al., 2018)	7	24,479,351	C/T	Ab (BLN)	0.0171	0.0296	3.096 (± 0.093) ^c	2.698 (± 0.107) ^d	2.701 (± 0.238)	2.56
				Viral load	0.0234	0.0731	47.04 (± 0.708)	49.04 (± 0.836)	50.34 (± 1.576)	2.12
				Ab (serum)	0.4531	0.7095	1.270 (± 0.062)	1.169 (± 0.082)	1.153 (± 0.188)	0.42
rs80794785 (Walker et al., 2018)	7	24,764,780	A/C	Ab (lung)	0.0024	0.0091	4.005 (± 0.094) ^c	4.459 (± 0.122) ^d	4.757 (± 0.254) ^d	5.84
				Ab (BLN)	0.0020	0.0064	2.742 (± 0.091) ^c	3.042 (± 0.120) ^a	3.639 (± 0.249) ^{b,f}	4.61
				Viral load	0.0028	0.0099	44.82 (± 1.185) ^c	46.55 (± 1.367)	49.79 (± 0.821) ^f	4.35
rs80947467 (Serao et al., 2014)	7	25,967,157	C/T	Ab (serum)	0.4312	0.6135	1.315 (± 0.133)	1.157 (± 0.153)	1.188 (± 0.079)	0.51
				Ab (lung)	0.0358	0.0004	3.809 (± 0.182) ^c	3.435 (± 0.215) ^g	4.586 (± 0.113) ^{d,h}	3.32
				Ab (BLN)	0.0961	0.0235	2.569 (± 0.190) ^a	2.368 (± 0.224) ^c	3.177 (± 0.122) ^{b,d}	1.62
rs80857355	7	26,149,917	A/G	Viral load	0.2695	0.1118	46.87 (± 0.905) ^a	49.21 (± 0.870) ^b	48.24 (± 0.862)	0.45
				Ab (serum)	<0.0001	0.0002	0.867 (± 0.090) ^{a,g}	1.267 (± 0.084) ^f	1.478 (± 0.084) ^h	9.53
				Ab (lung)	0.6202	0.2436	4.109 (± 0.137)	4.412 (± 0.130)	4.207 (± 0.130)	0.14
rs80881916	14	127,826,606	A/G	Ab (BLN)	0.2634	0.0827	2.963 (± 0.136)	3.137 (± 0.131) ^a	2.700 (± 0.129) ^b	0.54
				Viral load	0.2220	0.0554	48.38 (± 0.830) ^c	49.40 (± 0.792) ^d	46.92 (± 0.926)	0.56
				Ab (serum)	<0.0001	<0.0001	1.521 (± 0.084) ^{a,g}	1.219 (± 0.078) ^{b,e}	0.807 (± 0.097) ^h	12.62
ASG40106200	17	769,390	A/G	Ab (lung)	0.7370	0.6880	4.227 (± 0.132)	4.313 (± 0.124)	4.156 (± 0.152)	0.07
				Ab (BLN)	0.5569	0.0968	2.745 (± 0.131)	3.140 (± 0.126)	2.895 (± 0.149)	0.15
				Viral load	0.5932	0.7058	50.41 (± 1.457)	49.16 (± 0.914)	49.29 (± 0.996)	0.13
rs335100185	17	884,654	A/C	Ab (serum)	NA	0.4994	1.211 (± 0.162)	1.322 (± 0.090)	1.168 (± 0.102)	0.38
				Ab (lung)	0.8151	0.9714	4.237 (± 0.200)	4.250 (± 0.114)	4.281 (± 0.128)	0.03
				Ab (BLN)	0.0012	0.0044	3.488 (± 0.224) ^{a,g}	2.952 (± 0.125) ^b	2.645 (± 0.144) ^f	5.43
rs335100185	17	884,654	A/C	Viral load	0.8044	0.1088	47.70 (± 0.615)	49.39 (± 0.961)	45.70 (± 1.872)	0.02
				Ab (serum)	0.0708	0.1984	1.165 (± 0.045)	1.311 (± 0.099)	1.527 (± 0.219)	1.57
				Ab (lung)	<0.0001	0.0002	4.416 (± 0.078) ^c	3.886 (± 0.143) ^f	3.330 (± 0.299) ^f	7.49
rs335100185	17	884,654	A/C	Ab (BLN)	0.2357	0.4878	2.962 (± 0.083)	2.782 (± 0.152)	2.682 (± 0.315)	0.49
				Viral load	0.6615	0.2101	47.57 (± 1.316)	49.61 (± 1.023)	47.67 (± 0.667)	0.06
				Ab (serum)	0.5755	NA	1.435 (± 0.134)	1.248 (± 0.059)	1.169 (± 0.037)	0.68
rs335100185	17	884,654	A/C	Ab (lung)	0.0004	0.0013	3.535 (± 0.199) ^{a,g}	4.145 (± 0.151) ^d	4.386 (± 0.088) ^h	5.30
				Ab (BLN)	0.4258	0.3447	2.634 (± 0.208)	3.011 (± 0.159)	2.906 (± 0.088)	0.21

LSM = least square means; BLN = bronchial lymph node.

*a,b ($P < 0.1$); c,d ($P < 0.05$); e,f ($P < 0.01$); g,h ($P < 0.001$).

tenfold following stimulation with a dsRNA mimic (PolyI:C) or lipopolysaccharide and nearly 25-fold following stimulation with a set of pro-inflammatory cytokines (Ebstein et al., 2009). Based on this direct function in the innate immune response and high expression in the primary sites of PRRSV infection, alveolar macrophages, *LONRF1* is a likely candidate gene for lung Ab titer.

Targeted sequencing of *LONRF1* in a subset of samples ($n = 16$) revealed four coding SNPs (*rs695478970*, *rs321342913*, *rs318332242*, and *rs346383275*) including two nonsynonymous, *LONRF1 p.Ala114Pro* (*rs695478970*) and *LONRF1 p.Val435Ile* (*rs321342913*). However, both these substitutions are predicted by Ensembl to be tolerable in terms of maintaining protein function using the Sorting Intolerant From Tolerant tool (SIFT > 0.4). Reliable genotypes for *LONRF1 p.Ala114Pro* were unable to be obtained due the high GC content surrounding the SNP. However, we evaluated *LONRF1 p.Val435Ile* and the synonymous SNP, *rs318332242*, for their additive effects on lung Ab titer (14 dpi). While these two *LONRF1* SNPs were significant ($F < 12$; $P < 0.005$), their effects did not exceed the effects of the original QTL SNP, *ASGA0106200* and *rs335100185* ($F > 12$; $P < 0.0005$). Additional annotation of this region is required to further characterize genetic sources responsible for the variation in PRRSV Ab in the lung.

CONCLUSION

Genome-wide association analyses of pigs from two genetic lines experimentally infected with PRRSV revealed both novel and common genomic regions associated with PRRSV susceptibility traits. Specifically, this research identified a major region for PRRSV-specific antibody level in serum on SSC7 located near the *SLAII* locus. This region has been previously implicated in PRRSV and PCV2 infection, suggesting a potential role of this locus in susceptibility to multiple viral pathogens. Additionally, a novel genomic region on SSC17 was identified for PRRSV-specific antibody level in lung tissue. Genetic variants discovered in a positional candidate gene (*LONRF1*) within this region could play a potential role in navigating PRRSV-specific Ab production in lung tissue in response to PRRSV infection. This study highlights the role of host genetics in PRRSV susceptibility and the presence of a pleiotropic region (SSC7) involved in susceptibility to multiple viral pathogens. Potential future applications of this research could entail large commercial training and evaluation data sets, including

populations experiencing PRRSV outbreaks, and then applying the markers in genomic selection in nucleus population.

By increasing our understanding of how host genetics confers disease tolerance, we will be better able to combat difficult to manage viral pathogens like PRRSV, boosting overall animal welfare and the economic sustainability of the swine industry.

Conflict of interest statement. None declared.

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