ANIMAL GENETICS AND GENOMICS

Associations of natural variation in the CD163 and other candidate genes on host response of nursery pigs to porcine reproductive and respiratory syndrome virus infection

Qian Dong,[†,‡] Jenelle Dunkelberger,[†,‖] Kyu-Sang Lim,[†] Joan K. Lunney,[$] Christopher K. Tuggle,[†] Raymond R. R. Rowland,[¶] and Jack C. M. Dekkers[†,1]

[†]Department of Animal Science, Iowa State University, Ames, IA 50011, USA, [‡]Department of Epidemiology and Cancer Control, St. Jude Children's Research Hospital, Memphis, TN 38105, USA, [‖]Topigs Norsvin USA, Burnsville, MN 55337, USA, [$]USDA, ARS, BARC, Beltsville, MD 20705, USA, [¶]College of Veterinary Medicine, University of Illinois, Urbana, IL 61802, USA

[1]Corresponding author: jdekkers@iastate.edu

Abstract

Pigs with complete resistance to porcine reproductive and respiratory syndrome (**PRRS**) virus (**PRRSV**) have been produced by genetically knocking out the *CD163* gene that encodes a receptor of the PRRSV for entry into macrophages. The objectives of this study were to evaluate associations of naturally occurring single nucleotide polymorphisms (SNPs) in the *CD163* gene and in three other candidate genes (*CD169*, *RGS16*, and *TRAF1*) with host response to PRRSV-only infection and to PRRS vaccination and PRRSV/porcine circovirus 2b (**PCV2b**) coinfection. SNPs in the *CD163* gene were not included on SNP genotyping panels that were used for previous genome-wide association analyses of these data. An additional objective was to identify the potential genetic interaction of variants at these four candidate genes with a mutation in the *GBP5* gene that was previously identified to be associated with host response to PRRSV infection. Finally, the association of SNPs with expression level of the nearby gene was tested. Several SNPs in the *CD163*, *CD169*, and *RGS16* genes were significantly associated with host response under PRRSV-only and/or PRRSV/PCV2b coinfection. The effects of all SNPs that were significant in the PRRSV-only infection trials depend on genetic background. The effects of some SNPs in the *CD163*, *CD169*, and *RGS16* genes depend on genotype at the putative causative mutation in the *GBP5* gene, which indicates a potential biological interaction of these genes with *GBP5*. In addition, genome-wide association results for the PRRSV-only infection trials revealed that SNPs located in the *CDK5RAP2* or *MEGF9* genes, near the *TRAF1* gene, had suggestive effects on PRRS viral load, which indicates that these SNPs might contribute to PRRSV neuropathogenesis. In conclusion, natural genetic variants in the *CD163, CD169*, and *RGS16* genes are associated with resistance to PRRSV and/or PCV2b infection and appear to interact with the resistance quantitative trait locus in the *GBP5* gene. The identified SNPs can be used to select for increased natural resistance to PRRSV and/or PRRSV-PCV2b coinfection.

Key words: *CD163* gene, *CD169* gene, disease resistance, genetics, porcine reproductive and respiratory syndrome, swine

Abbreviations

dpi	days post infection
dpv	days postvaccination
FDR	false discovery rate
LD	linkage disequilibrium
PCV2b	porcine circovirus 2b
PHGC	PRRS Host Genetics Consortium
PRRS	porcine reproductive and respiratory syndrome
PRRSV	porcine reproductive and respiratory syndrome virus
QTL	quantitative trait loci
SNP	single nucleotide polymorphism
TCID50	tissue culture infectious dose

Introduction

Porcine reproductive and respiratory syndrome (**PRRS**) has caused dramatic economic losses in the swine industry throughout the world since the 1990s. The causative agent is an enveloped, positive, single-stranded RNA virus from the family Arteriviridae (Snijder et al., 2013) that has restricted tropism for porcine host cells of monocyte/macrophage origin. Pigs are the only known natural host of the PRRS virus (**PRRSV**; Lunney et al., 2016). The PRRS Host Genetics Consortium (**PHGC**) was established in 2007 to investigate the genetic basis of host response to PRRSV infection using large experimental infection studies of nursery pigs (Lunney et al., 2011). Results from the PHGC showed that the response of pigs to PRRSV infection is moderately heritable but highly polygenic (Waide et al., 2017), except for genotype at the guanylate binding protein 5 (*GBP5*) gene or region (Koltes et al., 2015), which was shown to have a major effect on both viremia and weight gain following PRRSV infection in nursery pigs (Boddicker et al., 2012, 2014a, 2014b). To our knowledge, no pigs with complete natural resistance to PRRS have been identified. In 2016, however, *CD163* gene knockout pigs were generated and found to be completely resistant to infection with PRRSV isolate NVSL 97-7895 (Whitworth et al., 2016). The *CD163* gene is a member of the scavenger receptor cysteine-rich (**SRCR**) superfamily and is a cellular receptor for PRRSV (Calvert et al., 2007). The *CD163* knockout pigs have a nonfunctional SRCR domain 5 (SRCR5) from exon 7, which demonstrates that SRCR5 in porcine CD163 is essential for PRRSV to infect macrophages (Burkard et al., 2017). Ma et al. (2017) found that a single base pair edit at position 561 in CD163 SRCR5 causes an arginine to alanine substitution that prevents PRRSV binding to the CD163 receptor during virus invasion. The CD163 receptor consists of nine extracellular SRCR domains and two proline-serine-threonine-rich domains. After deletion of SRCR 4–6 of CD163, the CD163 mutants were only expressed in HEK293T cells, but not on the cell surface, and thus none of the resulting HEK293T cells were infected by PRRSV (van Gorp et al., 2010). After deletion of SRCR 7–9 of CD163, none of the HEK293T cells were infected by PRRSV, although surface and intracellular expression of CD163 mutants were identified (van Gorp et al., 2010).

Johnsson et al. (2018) conducted pooled sequencing of the exons of the *CD163* gene of 35,000 pigs with different genetic backgrounds, and whole-genome sequencing of 3 pigs, with the aim to identify natural mutations in the *CD163* gene that may result in resistance of pigs to PRRSV infection, but no potential natural knockout variants of the *CD163* gene were found. However, Ren et al. (2012) identified three single nucleotide polymorphisms

(**SNPs**) in the *CD163* gene, of which two, c.2592A>G (also named A2552G) and c.2740C>A (also named C2700A), were associated with risk of PRRSV infection following a natural outbreak in a population of 524 approximately 1-y-old crossbred pigs (Duroc × [Landrace × Large White]). Using data from 47 (Landrace × Yorkshire) × Duroc pigs that were experimentally infected with the JA142 PRRSV strain, Lim et al. (2018) found significant associations of several SNPs in the *CD163* gene with PRRSV viremia and weight gain at 21 days post infection (**dpi**), and with average viremia at 3, 14, 21, and 28 dpi, including the c.2509G>C, c.2638A>G, and c.3534C>T polymorphisms. The latter SNP is located in the 3′-UTR region of the *CD163* gene and was also found by Wang et al. (2012) to be significantly associated with IgG content in blood in a population of 128 healthy pigs from different breeds and crosses. The two SNPs in the *CD163* gene that were identified by Ren et al. (2012) were, however, not found to be significant in the study of Lim et al. (2018). Interestingly, Lim et al. (2018) found both the c.2509G>C and the c.3534C>T SNPs to have significant interaction effects with genotype at the WUR10000125 (WUR) SNP, which has previously been identified to have significant associations with PRRS viral load and weight gain after PRRSV infection in the PHGC trials (Boddicker et al., 2012). The WUR SNP is in very high linkage disequilibrium (**LD**) with the putative causative mutation for this association in the *GBP5* gene (Koltes et al., 2015).

The SNP panel that was used to genotype the pigs in the PHGC trials did not include any SNPs in the *CD163* gene, because the *CD163* gene was not included in porcine genome builds prior to the current 11.1 build (http://www.ensembl.org/Sus_scrofa/Info/Index). The same holds for SNPs in several other candidate genes important for host response to viral infection, including *CD169*, *RGS16*, and *TRAF1*, although these genes were included in the earlier 10.2 build of the porcine genome.

Van Breedam et al. (2010) postulated that CD169 is a required receptor on the surface of macrophages for PRRSV attachment and internalization into macrophages, based on binding with sialic acid on the PRRSV surface in vitro. However, Prather et al. (2013) showed that *CD169* gene knockout pigs developed PRRSV viremia to a similar degree as wild-type pigs after PRRSV infection, which demonstrated that CD169 is not required for PRRSV infection. However, in addition to having a role in pathogen uptake into macrophages, CD169 has other roles in the immune response. Specifically, CD169 can promote CD8+ cytotoxic T cell activity through cross-presentation with CD8α+ dendritic cells (classical DC1, cDC1; van Dinther et al., 2018; Uchil et al., 2019). The binding of CD169 to sialic acid ligands on other cells, such as cDC1 and B cells, participates in anti-pathogen immune response by activating T and B cells (reviewed in Neill et al., 2012). Ren et al. (2012) identified three SNPs in the *CD169* gene and found two to be associated with risk of PRRSV infection in a population of 524 pigs following a natural PRRS outbreak. Wang et al. (2012) found another non-synonymous SNP, c.878A>G, in exon 3 of the *CD169* gene to be associated with white blood cell count in peripheral blood in a mixed breed population of 128 healthy pigs. Here, we evaluated associations of these same four SNPs in the *CD169* gene with host response to PRRSV infection in the PHGC trials.

The regulator of G protein signaling 16 (*RGS16*) gene produces a GTPase-activating protein that can induce T cell migration and activation (Webster et al., 2014). RGS16 can interact with ORF3 of PCV2 and plays a role in ORF3 translocation to the cell nucleus (Lovgren et al., 2009). ORF3 of PCV2 is involved in its pathogenesis and contributes to the spread of PCV2 in cell culture through apoptosis (Karuppannan, 2011). PCV2 is a

non-enveloped, single-stranded, circular DNA virus (Todd et al., 1991) and is the causative agent of postweaning multisystemic wasting syndrome (Harding and Clark, 1997). Lim et al. (2017) found two SNPs in the 5′ upstream region of the *RGS16* gene to be associated with PCV2 viremia at 10 wk of age in 142 naturally infected pigs that were not vaccinated for PCV2. Therefore, we set out to validate the effects of these two *RGS16* SNPs on PCV2 viral load in the PHGC PRRSV/porcine circovirus 2b (**PCV2b**) coinfection trials reported by Dunkelberger et al. (2017a). Both PCV2 and PRRSV can suppress the host immune defense system and persist asymptomatically in pigs, which can cause secondary infections in affected susceptible pigs. Therefore, we also tested the effect of the two SNPs in the *RGS16* gene on PRRS viral load in the PHGC PRRSV-only infection trials reported by Boddicker et al. (2014a).

The TNF receptor-associated factor 1 (TRAF1) protein can interact with the Toll/IL-1 receptor (TIR) domain-containing adaptor protein (TRIF), which might negatively regulate the TRIF- and Toll-like receptor-3 (TLR3)-mediated signaling pathway, which plays an essential role in mediating antiviral innate immune response (Su et al., 2005). Su et al. (2005) found a non-cleavable *TRAF1* mutant, *TRAF1* (D163A), that lost its inhibitory role on TRIF signaling. Although *TRIF* is involved in TLR3-mediated signaling and TLR3 protein can recognize double-strand (ds) RNA as a pathogen-associated molecular patterns, Guo et al. (2018) identified dsRNA in cells of pigs during the persistent stage of PRRSV infection. In this study, we evaluated two SNPs in the *TRAF1* gene.

Against this background, the objective of this study was to identify associations of natural variation in the *CD163*, *CD169*, *RGS16*, and *TRAF1* genes on host response of nursery pigs to PRRSV-only infection and to coinfection with PRRSV and PCV2b with or without prior vaccination with a PRRS-modified live virus vaccine.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved all experimental protocols for the animal trials used herein.

Experimental design

Data and DNA used for this study were from the PRRSV infection and PRRSV-PCV2b coinfection PHGC trials. A detailed description of the PHGC PRRSV-infection trials (PHGC 1–8) is in Boddicker et al. (2014a). Briefly, 8 groups of ~200 commercial crossbred piglets (PHGC trials 1–8) from one of six breeding companies were shipped to Kansas State University (KSU, Manhattan, KS) at weaning. After 1 wk of acclimation, all pigs were inoculated with a 10^5 tissue culture infectious dose (TCID50) of the NVSL PRRSV isolate, with approximately half of the dose injected intramuscularly and the rest intranasally. Blood samples were collected from each pig at 0, 4, 7, 11, 14, 21, 28, 35, and 42 dpi. Body weight was recorded weekly, starting at 0 dpi and most pigs were euthanized at 42 dpi.

A detailed description of the two coinfection trials, PHGC 16 (n = 199) and 20 (n = 197), is in Dunkelberger et al. (2017a). Briefly, pigs were commercial Large White × Landrace crossbred barrows from one genetic source and one high-health multiplier farm. Piglets were pre-selected based on genotype at the WUR SNP, with approximately 50% AA and 50% AB. After shipping to Kansas State University, pigs were randomly assigned to one of two rooms and balanced by WUR genotype. After 3–4 d of acclimation, pigs in one room were vaccinated with a 2 mL dose of a PRRS-modified live virus vaccine (Ingelvac PRRS, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO; GenBank accession no. AF159149). After 28 days postvaccination (**dpv**), all pigs in both rooms were coinfected on 0 dpi with a 2 mL dose of 10^5 TCID50 PRRSV (isolate KS62; GenBank accession no. KM035803) and $10^{3.6}$ TCID50 PCV2b (GenBank accession no. JQ692110), which were administered both intranasally and intramuscularly (Niederwerder et al., 2015). All pigs were euthanized at 42 dpi. Blood samples were collected on vaccinated pigs at 0, 4, 7, 11, 14, and 21 dpv and on all pigs at 0, 4, 7, 11, 14, 21, 28, 35, and 42 dpi. All pigs were weighed weekly, starting at 0 dpv.

Genotypes

All pigs from the PRRSV-infection trials were genotyped using the Illumina Porcine SNP60 BeadChip (Ramos et al., 2009) and pigs from the coinfection trials were genotyped using the GeneSeek-Neogen Porcine SNP80 BeadChip (Dunkelberger et al., 2017b). Fixed SNPs and SNPs with a genotyping call rate less than 0.85 were removed, while genotypes with a gene call score lower than 0.3 were set to missing.

For the purposes of this study, DNA samples from all pigs were genotyped for an additional 27 SNPs in the four candidate genes (19 in *CD163*, 4 in *CD169*, and 2 in both *TRAF1* and *RGS16*) using a custom-designed MassARRAY platform (Agena Bioscience, San Diego, CA, USA) according to the manufacturer's instructions. Most of these SNPs were previously identified to be associated with host response to PRRSV or PCV2 (*RGS16* only) infection (Ren et al., 2012; Wang et al., 2012; Lim et al., 2017, 2018). All pigs were genotyped for two SNPs in the *TRAF1* gene that were identified in our previous tonsil RNA-seq data (Dong et al., 2021). Details on the 27 SNPs are in Supplementary Table S1 annotated based on build 11.1 of the pig genome (Ensembl).

LD between SNPs in each gene was quantified based on r^2, using Haploview 4.2 (Barrett et al., 2005), both across trials and by genetic source. SNPs within a gene that were in complete LD across all PRRS trials or across the two coinfection trials were merged.

Phenotypes

For the PRRSV-infection trials, two phenotypes were analyzed: PRRS viral load, which was calculated as the area under the curve of $\log_{10}$ viral copies/mL of serum from 0 to 21 dpi, following Boddicker et al. (2012) and weight gain after infection, which was calculated as the difference in body weights at 42 and 0 dpi. Viral load represents an overall measure of the immune response after infection, which previous studies have shown to be more heritable than viremia at individual time points or than specific parameters of the viremia curve (Boddicker et al., 2012; Hess et al., 2016). For the coinfection trials, the host response traits described by Dunkelberger et al. (2017a) were analyzed: PRRS viral load postvaccination from 0 to 28 dpv (Vaccination viral load), PRRS viral load post coinfection, and PCV2b viral load, all calculated as area under the curve of the $\log_{10}$ of PRRSV RNA or PCV2b DNA copies/mL of serum. Growth rate postvaccination and post coinfection were calculated as the regression of body weight on age from 0 to 28 dpv and from 0 to 42 dpi, respectively. The same trait recorded on vaccinated vs. non-vaccinated pigs were treated as separate genetic traits in the association analyses.

Association analyses for the PRRSV-infection trials

Associations of the 27 genotyped candidate gene SNPs with viral load and weight gain in the PRRSV-only trials were analyzed

using three methods: single SNP analyses and Bayesian variable selection analyses. Single SNP analyses were similar to those described by Waide et al. (2017), using ASReml 4 (Gilmour et al., 2015). Briefly, the following linear mixed model was used to test the association of each SNP with PRRS viral load and weight gain by fitting the genotype at one SNP at a time as a fixed effect in the following model (Model 1):

$$Y_{ijklmnop} = \mu + \text{Trial}_j + \text{Parity}_{k(j)} + \text{WUR}_l + \text{Sex}_m + \text{SNP}_n \\ + \text{SNP}_n * \text{Trial}_j + \beta_1 * \text{InWt}_i + \beta_2 * \text{InAge}_i \\ + \text{Animal}_i + \text{Litter}_o + \text{Pen}_{p(j)} + e_{ijklmnop},$$

where $Y_{ijklmnop}$ is the observed phenotype (PRRS viral load or weight gain); Trial_j is the fixed effect of the jth trial (1–8); Parity_k is the fixed effect of parity of sow nested within trial (20 levels); WUR_l is the fixed effect of WUR SNP genotype (AA, AB, or BB); Sex_m is the fixed effect of sex (male or female); SNP_n is the fixed effect of the nth genotype (0, 1, or 2) at the fitted SNP; β_p is the partial regression coefficients for the covariates of initial weight (InWt; $P = 1$) and initial age (InAge; $P = 2$); Animal_i is the random genetic effect of the ith individual, assumed distributed $\sim N(0, G\sigma_a^2)$, with G equal to the genomic relationship matrix constructed using all genotyped SNPs (60K plus the 27 candidate gene SNPs), and σ_a^2 is the additive genetic variance; Litter_o is the random litter effect (400 levels), assumed to be distributed $\sim N(0, I \sigma_l^2)$; and Pen is the random effect of pen nested within trial (97 levels), assumed to be distributed $\sim N(0, I \sigma_p^2)$. Since the effect of trial (environmental effect) was confounded with genetic source in the experimental design, we assumed that pigs from different genetic sources were not related when constructing G. The Benjamini–Hochberg method (Benjamini and Hochberg, 1995) was used to control false discovery rate (**FDR**) across the 27 analyzed SNPs but separately for each trait.

For the Bayesian variable selection analyses, effects of genotypes at all SNPs (60K plus the 27 candidate gene SNPs and the putative causal SNP rs340943904 in *GBP5*) were fitted simultaneously as random allele substitution effects using the Bayes-B method (Habier et al., 2011), as implemented in JWAS software (Cheng et al., 2018). The linear mixed model was the same as used for the single SNP analysis (Model [1]) but without WUR genotype as a fixed effect and with the effects of SNP and animal replaced by $\sum_n^N z_{ni}\alpha_n\delta_n$, where z_{ni} is the vector of the genotype covariate for SNP n ($n = 1$ to N) for individual i based on the number of B alleles using Illumina's genotype calling (coded 0, 1, 2, or equal to the average for the individual's genetic background (i.e., genetic source) for missing genotypes); α_n is the allele substitution effect for SNP n and δ_n is the indicator for whether SNP n was included ($\delta_n = 1$) or excluded ($\delta_n = 0$) in the model for a given iteration of the Markov Chain Monte Carlo run. The prior probability of $\delta_n = 0$ was set equal to $\pi = 0.99$. A total of 50,000 iterations were run for each analysis, with the first 5,000 iterations discarded as burn-in. Genomic regions associated with traits were identified based on the genetic variance explained by each non-overlapping 1 Mb window, based on build 11.1 of the pig genome (Ensembl).

Bivariate single SNP analyses for coinfection trials

Associations of the candidate gene SNPs with traits evaluated postvaccination and post coinfection were analyzed using the bivariate animal models described by Dunkelberger et al. (2017b) to allow for differences in the genetic control of a trait, depending on prior PRRS vaccination. The linear mixed models allowed estimation of the effect of each SNP, one at a time, averaged across PRRS vaccination status, and the interaction

effect between SNP genotype and vaccination status on PRRS and PCV2b viral load post coinfection and growth rate postvaccination or post coinfection. Fixed effects fitted in the model included trial and WUR genotype, covariates of initial weight, age, and PCV2b viremia at 0 dpi, and the random effects of pen within trial, litter, and animal to account for random environmental, common environmental, and genetic effects, respectively, with bivariate distributions equivalent to the univariate distributions of Model 1, but allowing for covariances between the two traits analyzed (with and without vaccination).

Associations of SNPs in candidate genes with expression of the corresponding genes

Expression of the *CD163*, *CD169*, and *RSG16* genes was measured in blood at multiple time points after vaccination and coinfection in the coinfection trials, using QuantSeq 3′ mRNA-Seq (Dong, 2019). Blood RNA levels from two sets of pigs from PHGC 16 (PHGC 16.1 and 16.2) and from one set of pigs from PHGC 20 were used. For PHGC 16.1, 190 blood samples collected on 28 piglets from 7 litters that had one piglet for each vaccination status by WUR genotype combination (AA-vaccinated, AB-vaccinated, AA-non-vaccinated, and AB-non-vaccinated) were selected. For PHGC 16.2, 288 blood samples on 49 piglets from 16 litters with at least two of the four vaccination status by WUR genotype combinations were selected. For PHGC 20, 216 blood samples on 30 piglets from 9 litters with at least three of the four vaccination status by WUR genotype combinations were selected. Total RNA was isolated using Preserved Blood RNA Purification Kit I (Norgen, Canada) according to the manufacturer's protocol. RNA concentration, purity and integrity were first measured with a NanoDrop ND-1000 UV-vis Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) and then using the RNA Nano 6000 Assay kit on the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). As a measurement of the quality of the RNA in a sample, the RNA integrity number (RIN) of the extracted RNA was determined for each sample by the 2100 Bioanalyzer (Agilent Technologies) using the Eukaryote total RNA 6000 Nano kit. RNA-seq libraries were generated from ~500 ng of total RNA from each sample, using the QuantSeq 3′ mRNA-Seq Library Prep Kit FWD for Illumina, following the manufacturer's protocol. For PHGC 16.2 and 20, the RNA Removal Solution-Globin Block kit for *Sus scrofa* (commercially available as RS-GBSs: Lexogen Cat. No. 071; Lim et al., 2019) was used to reduce the presence of HBA and HBB in the library. For PHGC 16.1, the regular Removal Solution without globin block was used because the globin block kit was not available yet at the time these samples were sequenced. Libraries from piglets from the same litter were assigned to the same plate and multiplexed to 96 samples. Each plate was loaded on two lanes of the Illumina HiSeq 3000 Sequencing System (Illumina, USA) and 50 bp single end reads sequenced.

The raw RNA sequencing data were processed using a pipeline with Bbduk (https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/bbduk-guide/) to remove poly-A tails, adapter sequences, and low-quality bases, FastQC (Version 0.11.3) for checking read quality before and after trimming (Andrew et al., 2010), and STAR (Version 2.5.3; Dobin et al., 2013) for read alignment. To avoid multiple mapping issues of hemoglobin genes based on the Sus Scrofa 11.1 reference genome, one of two similar exon sequences that are present in 11.1 within *HBA* (ENSSSCG00000007978) and *HBB*-like (ENSSSCG00000014727) were masked. For gene annotation, the Ensembl pig 11.1 gene annotation was used. Any reads from the *HBA* (ENSSSCG00000007978) and

HBB (ENSSSCG00000014725) genes were filtered out before further analyses. Samples that had a 75th percentile read counts across all genes that was less than or equal to two were removed. Genes that had average read counts across all remaining samples that were less than or equal to two were excluded from further analysis.

Gene expression counts (on $\log_2$ scale of [count per million+1]) of the *CD163*, *CD169*, and *RSG16* genes were extracted from the three resulting data sets and combined for single SNP association analyses with SNPs in the *CD163*, *CD169*, and *RSG16* genes (the SNPs in *TRAF1* gene were not included in this analysis because the two SNPs in *TRAF1* were in complete LD and all but two pigs were of one homozygous genotype in PHGC 16 and 20). The analyses were conducted separately for time points before and after coinfection, using a mixed linear model in SAS 9.4 (SAS Institute, Cary, NC). The full model included the fixed effects of genotype at a SNP in the *CD163*, *CD169*, and *RSG16* genes (one at a time), WUR genotype, PRRS vaccination status, dpv (or dpi), RNA integrity number, and trial (PHGC 16 or 20), and the random effect of litter. For each data set (before and after coinfection), a compound symmetry covariance structure was fitted for residuals to account for the dependence of gene expression in samples from the same pig across time points. We did not conduct multiple test correction because the number of SNPs analyzed was limited (9, 4, and 2 for the *CD163*, *CD169*, and *RSG16* genes, respectively, after merging SNPs in complete LD and excluding SNPs that were fixed in the coinfection trials).

Results

Linkage disequilibrium

Pigs from eight PRRSV infection and from two PRRSV/PCV2b coinfection trials of ~200 pigs each were genotyped for 27 SNPs in four candidate genes (19 in *CD163*, 4 in *CD169*, and 2 each in both *TRAF1* and *RGS16*; Supplementary Table S1). Pigs were from six different commercial line crosses (see Boddicker et al., 2014a; Dunkelberger et al., 2017a). Pigs from PRRSV-only trials 1, 2, and 3, and from the two coinfection trials were Landrace × Yorkshire crossbred pigs from the same breeding company. Pigs from the PRRSV-only and the coinfection trials had previously been genotyped using the 60K and 80K SNP panels, respectively, which did not include SNPs in the *CD163*, *CD169*, *TRAF1*, or *RGS16* genes.

Results for LD between genotyped SNPs in the *CD163* gene are shown in Figure 1, both across the eight PRRSV-only trials (Figure 1A) and across the two coinfection trials (Figure 1B). Results for LD for each of the six genetic sources used in the PRRSV-only trials are shown in Supplementary Figure S1. SNPs 6, 16, and 17 were fixed in the PRRSV-only trials, while SNPs 2, 6, and 17 were fixed in the coinfection trials, and are, therefore, not shown in Figure 1. In the PRRSV-only trials, SNPs 4, 9, and 14 were in complete LD, so they were combined into one SNP called "SNP 4_9_14"; similarly, SNPs 11, 12, and 13 were merged into SNP 11_12_13. In the coinfection trials, SNPs 1, 4, 9, and 14 were in complete LD and merged into SNP 1_4_9_14, while SNPs 5, 11, 12, and 13 were combined into SNP 5_11_12_13. The presence of high LD between *CD163* SNPs was fairly consistent across the PRRSV-only trials and for the coinfection trials. However, because there were some differences in LD between genetic sources (Supplementary Figure S1), the interaction between genetic source and SNP genotype was tested in the association analyses of the PRRSV-only trials.

For the *CD169* SNPs, the LD was 0.6 between SNPs 1 and 4, and 0.4 between SNPs 3 and 4, while SNP 2 was in very low LD with the other three SNPs across pigs in both the PRRSV-only and the coinfection trials (results not shown). The LD between SNPs 1 and 3 was 0.2 and 0.4 in the PRRSV-only and in the coinfection trial animals, respectively. The two SNPs in the *TRAF1* gene were in complete LD in both the PRRSV-only and the coinfection trial pigs. The LD between the two SNPs in the *RGS16* gene were 0.3 and 0.8 in the PRRSV-only and in the coinfection trial populations, respectively.

SNP associations with host response in the PRRSV-only infection trials

In the PRRSV-only trials, five *CD163* SNPs and one *CD169* SNP were significantly associated with PRRS viral load (FDR < 0.1; Table 1); viral load was defined as area under the curve of the log of PRRS viremia in blood from 0 to 21 dpi (Boddicker et al., 2012). None of the evaluated SNPs had significant associations with weight gain. The five significant *CD163* SNPs clustered into two groups of SNPs that were in high LD with each other: 5 and 11_12_13, and 15, 18, 19, and had similar estimates of effects on PRRS viral load. The LD between these two clusters of SNPs ranged from 0.13 to 0.27 in the PRRSV-only trials (Figure 1A).

All significant SNPs in Table 1 (FDR < 0.1) had significant interaction effects with genetic background, that is, genetic source, for PRRS viral load but not for weight gain ($P > 0.1$). The significant interaction effects for viral load resulted from a difference in the genotype effects in trials 1–3 (the same genetic source) vs. the combined trials 4–8 (each from a different genetic source); interactions with genetic source were not significant for trials 4, 5, 6, 7, and 8. Estimates of the effect of these six SNPs on PRRS viral load for trials 1–3 vs. 4–8 are shown in Table 2 ($P < 0.1$). For all *CD163* SNPs, heterozygotes had significantly lower PRRS viral load than homozygotes for the major alleles in trials 1–3 but this difference was not significant for trials 4–8 (Table 2). Homozygotes for the minor alleles were not significantly different from heterozygotes for trial 1–3 but had significantly higher PRRS viral load for *CD163* SNPs 5 and 11_12_13 for trials 4–8. For *CD169* SNP 2, the direction of the effect was opposite between trials 1–3 and 4–8, with homozygotes for the major allele having the highest PRRS viral load for trial 1–3 but significantly lower PRRS viral load than homozygotes for the minor allele for trials 4–8 (Table 2).

The interaction effect between SNP genotype and genotype at the tag marker for *GBP5* (WUR) was tested for PRRS viral load and weight gain but was only suggestive for weight gain for SNP 2 in the *RGS16* gene in the PRRSV-only infection trials (Figure 2A; nominal $P = 0.08$, which was not significant after multiple test correction), and for weight gain for *CD163* SNPs 5 and 11_12_13 in trials 1–3 (Table 3). For trials 1–3, SNP effects were significant for weight gain for the AB genotype at WUR but not for the AA genotype at WUR, with heterozygotes at SNP 5 and 11_12_13 being favorable for both weight gain (only for the AB genotype at WUR) and for PRRS viral load (for both WUR genotypes; Table 3). For SNPs 15, 18, and 19, the heterozygotes had lower PRRS viral load than homozygotes for the major alleles for both WUR genotypes, consistent with the main effect results in Table 2, but heterozygotes had greater weight gain only for WUR genotype AB, although this was significant only for SNP 19 (Table 3).

The genome-wide association study results from the Bayesian variable selection analyses are shown in Figure 3. The *GBP5* window on chromosome 4 includes the putative causal SNP in the *GBP5* gene and the WUR SNP, which explained the largest proportion of genetic variance for both PRRS viral load (17.8%)
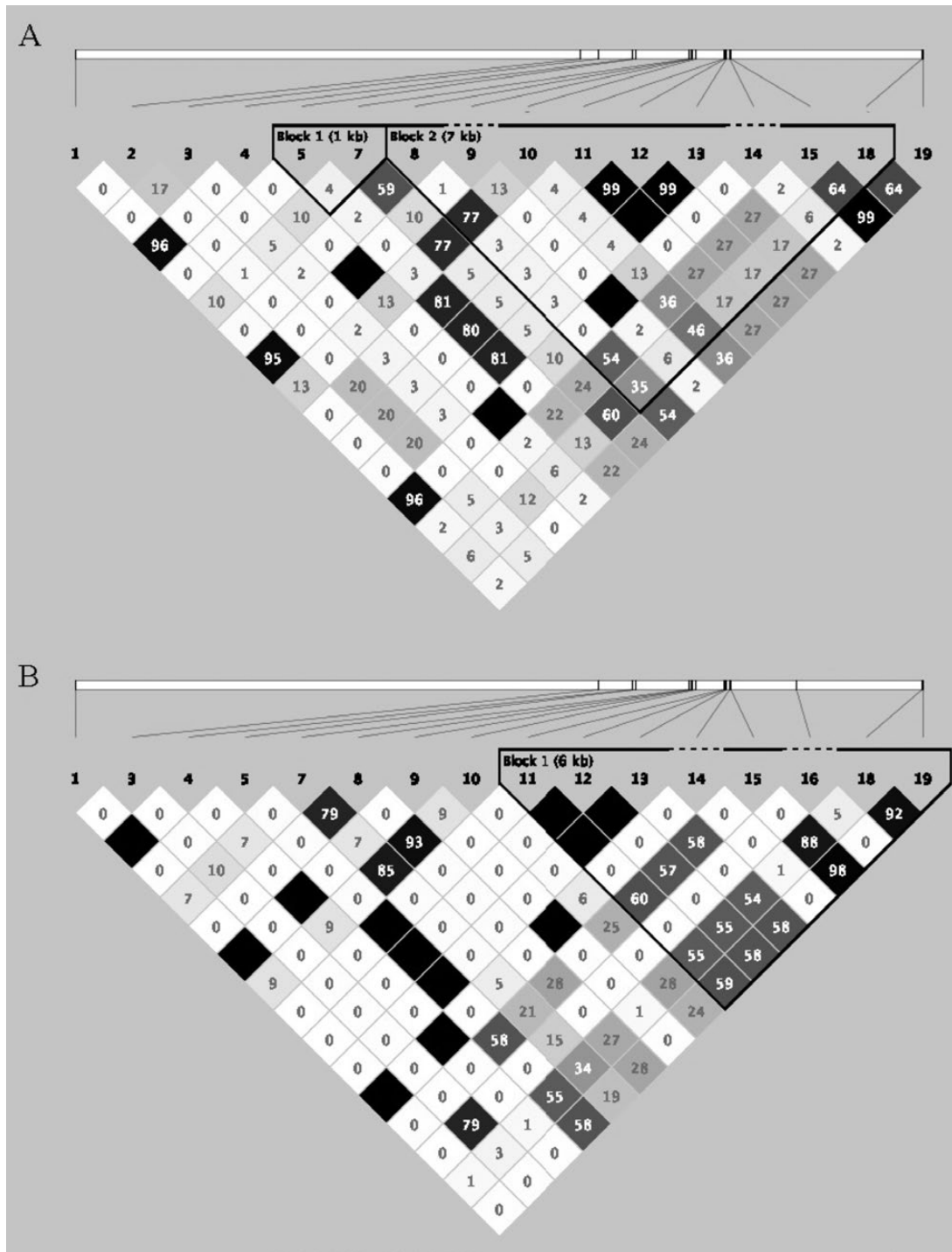
Figure 1. Linkage disequilibrium (*r²*) plot of the genotyped SNPs in the CD163 gene across the PRRSV-only infection trials 1 through 8 (A) and across the coinfection trials 16 and 20 (B). Black squares signify *r²* = 100% and white squares signify *r²* = 0%. PRRSV, PRRS virus; SNP, single nucleotide polymorphism.

and weight gain (9.8%), consistent with previous analyses of these data (Waide et al., 2017). The CD163 and TRAF1 windows were the top two windows after the GBP5 window for PRRS viral load, although they explained less than 2% of the genetic variance (1.1% and 0.9%, respectively). However, in our single SNP analyses, the candidate SNP in the TRAF1 gene was not significantly associated with PRRS viral load (Table 1), thus the association of this window with PRRS viral load was due to SNPs that were external to the TRAF1 gene (Supplementary Table S2 and Supplementary Figure S2). For weight gain, a 1 Mb window that was 7 Mb downstream from the CD163 window was the top window after the GBP5 window, explaining 2.4% of genetic variance.

Table 1. Association of SNPs in four candidate genes with host response to PRRSV-only infection

Gene	SNP[1]	Alleles[2]	No. of animals with genotype	Minor allele frequency	Least square means (SE) for PRRS viral load[3] by genotype			Nominal P-value	False discovery rate
					0	1	2[4]		
CD163	1	C/T	1,412	0.04	105.3 (1.0)	104.6 (1.2)	112.5 (5.1)	0.22	0.51
	2	C/A	1,414	0.03	105.3 (1.0)	106.0 (1.3)	112.1 (6.1)	0.41	0.68
	3	C/T	1,413	0.08	105.2 (1.0)	105.4 (1.1)	109.1 (2.6)	0.27	0.54
	4_9_14	G_G_C/A_A_T	1,420	0.05	105.3 (1.0)	104.4 (1.2)	112.3 (5.1)	0.14	0.44
	5	T/C	1,415	0.10	105.7 (1.0)a	102.8 (1.1)b	108.0 (2.5)a	0.000001	0.00002
	6	G/A	1,414	0.00	105.3 (1.0)	111.2 (4.4)	100.1 (4.4)	0.18	0.50
	7	C/G	1,411	0.30	105.6 (1.0)	105.2 (1.0)	105.2 (1.2)	0.65	0.84
	8	A/G	1,419	0.21	105.5 (1.0)	105.2 (1.0)	105.2 (1.4)	0.75	0.85
	10	C/A	1,411	0.25	105.6 (1.0)	105.0 (1.0)	105.2 (1.3)	0.43	0.68
	11_12_13	C_G_C/T_A_A	1,420	0.12	105.7 (1.0)a	103.3 (1.1)b	108.2 (2.2)a	0.00001	0.0001
	15	T/C	1,406	0.32	106.0 (1.0)a	104.8 (1.0)b	104.7 (1.2)b	0.01	0.04
	16	G/A	1,412	0.07	105.3 (1.0)	105.6 (1.2)	105.5 (1.6)	0.91	0.92
	17	C/A	1,416	0.00	105.4 (1.0)	112.6 (6.1)	.	0.23	0.51
	18	T/C	1,408	0.42	106.3 (1.0)a	104.8 (1.0)b	104.9 (1.1)b	0.01	0.04
	19	A/G	1,409	0.32	106.0 (1.0)a	104.8 (1.0)b	104.8 (1.2)ab	0.01	0.04
CD169	1	G/A	1,410	0.24	105.4 (1.0)	105.3 (1.0)	106.1 (1.4)	0.72	0.85
	2	G/T	1,386	0.08	105.6 (1.0)a	104.0 (1.1)b	103.1 (2.4)ab	0.02	0.07
	3	C/A	1,418	0.45	105.4 (1.0)	105.3 (1.0)	105.5 (1.0)	0.92	0.92
	4	C/T	1,411	0.35	105.5 (1.0)	105.2 (1.0)	105.3 (1.1)	0.77	0.85
TRAF1	1_2	C_C/G_T	1,413	0.10	105.5 (1.0)	104.5 (1.3)	105.8 (2.7)	0.53	0.73
RGS16	1	A/G	1,412	0.42	105.7 (1.0)	105.3 (1.0)	104.8 (1.1)	0.30	0.55
	2	C/T	1,413	0.38	105.7 (1.0)	105.2 (1.0)	105.2 (1.1)	0.51	0.73

[1]SNPs are labeled based on reference Supplemental Table S1. SNPs with multiple numbers indicate "merged" SNPs based on LD.
[2]The second letter indicates the minor allele.
[3]PRRS viral load was calculated as the area under the curve from 0 to 21 dpi.
[4]Homozygotes for the minor allele.
[a,b]Estimates with different letter superscripts within a SNP are significantly different at a nominal $P < 0.1$.
PRRS, porcine reproductive and respiratory syndrome; PRRSV, PRRS virus; LD, linkage disequilibrium.

Table 2. Estimates (SE) of the genotype effects at SNPs in the CD163 and CD169 gene that interact with genetic background[1] on PRRS viral load[2] in the PRRSV-only infection trials

Gene	SNP	Alleles[3]	Estimates (SE) of SNP genotype effects for trials 1+2+3			Estimates (SE) of SNP genotype effects for trials 4+5+6+7+8			Nominal P-value[5] of genetic background by SNP interaction
			0	1	2[4]	0	1	2	
CD163	5	T/C	0[a]	−5.7 (0.9)[b]	−2.0 (3.8)[ab]	0.8 (0.8)[a]	0[a]	5.9 (2.8)[b]	5.4×10^{-9}
	11_12_13	C_G_C/T_A_A	0[a]	−5.1 (0.9)[b]	−1.7 (3.8)[ab]	0.5 (0.7)[a]	0[a]	5.4 (2.3)[b]	6.4×10^{-8}
	15	T/C	0[a]	−2.4 (0.7)[b]	−5.6 (2.0)[b]	0.4 (0.5)[a]	0[a]	0.2 (0.8)[a]	1.3×10^{-3}
	18	T/C	0[a]	−2.4 (0.7)[b]	−2.0 (1.8)[ab]	0.7 (0.8)[a]	0.03 (0.6)[a]	0[a]	6.1×10^{-3}
	19	A/G	0[a]	−2.5 (0.7)[b]	−5.2 (2.1)[b]	0.4 (0.5)[a]	0[a]	0.3 (0.8)[a]	1.6×10^{-3}
CD169	2	G/T	6.8 (2.8)[a]	4.1 (3.0)[b]	0[b]	0[a]	−1.0 (0.7)[ab]	4.3 (3.6)[b]	4.8×10^{-3}

[1]Pigs from trials 1–3 originated from the same genetic source, whereas pigs from trials 4–8 each originated from a different genetic source.
[2]PRRS viral load, calculated as the area under the curve from 0 to 21 dpi.
[3]The second letter indicates the minor allele.
[4]Homozygotes for the minor allele.
[5]Nominal P <0.01.
[a,b]Estimates with different letter superscripts within a SNP and trial set are significantly different at a nominal P < 0.1.
PRRS, porcine reproductive and respiratory syndrome; PRRSV, PRRS virus.

SNP associations with host response in the coinfection trials

In the PRRSV and PCV2b coinfection trials, none of the SNPs in the *CD163* gene had a significant main effect association with PRRS viral load, even at an FDR threshold of 0.4. However, SNPs 5 and 8 showed suggestive (nominal P of 0.05 and 0.04, respectively) interaction effects with WUR genotype on PRRS viral load following first exposure (primary PRRS viral load, i.e., postvaccination or post coinfection without vaccination; Figure 2B and 2C). Note that SNP 5 also showed an interaction effect with WUR genotype on PRRS viral load in the PRRSV-only trials; in both sets of trials, heterozygotes for SNP 5 had higher primary PRRS viral load than homozygotes for the major allele for animals with WUR genotype AB, while the effect of SNP 5 was not present (PRRSV-only) or in the opposite direction (coinfection trials) for pigs with the AA genotype at WUR. SNP 8 did not have a significant interaction effect with WUR genotype in the PRRSV-only trials (P = 0.87 for PRRS viral load; 0.96 for weight gain). SNP 7 in the *CD163* gene was suggestively associated with PCV2b viral load post coinfection from 0 to 42 dpi (PCV2b viral load; FDR = 0.35, P = 0.04), with growth rate postvaccination (growth rate postvaccination, FDR = 0.32, P = 0.06), and with growth rate post coinfection (growth rate post coinfection, FDR = 0.38, P = 0.11; Table 4). Compared with homozygotes for the major allele, heterozygotes at this SNP tended to have higher PCV2b viral load and lower growth rate following coinfection, but higher growth rate following PRRS vaccination. SNP 7 did not have significant effects on either viral load or weight gain in the PRRSV-only trials.

For the *CD169* gene, genotype at SNP 1 was suggestively associated with PCV2b viral load (FDR = 0.35, nominal P = 0.03; Table 4) and had significant interaction effects with WUR genotype on growth rate postvaccination and with growth rate post coinfection (P = 0.09 and 0.03, Figure 2D and 2E). Pigs that were homozygous for the minor allele at SNP 1 had greater PCV2b viral load and tended to have lower growth rate following coinfection (Table 4), especially for pigs with the AB genotype at WUR (Figure 2E). Genotype at SNP 3 in the *CD169* gene was suggestively associated with growth rate post coinfection (FDR = 0.38, P = 0.11). Finally, SNPs 2 and 4 in the *CD169* gene had significant interaction effects with PRRS vaccination status for growth rate postvaccination (P = 0.07) and for PCV2b viral load (P = 0.02), respectively (Figure 2F and 2G).

For the *RGS16* gene, SNP 2 had a significant interaction effect with WUR genotype on growth rate postvaccination (P = 0.01, Figure 2H), with the difference in growth rate between pigs with the AB vs. AA genotypes at WUR increasing with the number of minor alleles carried at SNP 2, starting with essentially no effect for pigs that were homozygous for the major allele at SNP 2. The genotyped SNPs in the *TRAF1* gene were not significantly associated with any of the traits evaluated from the coinfection trials.

SNP associations with expression of the *CD163*, *CD169*, and *RSG16* genes

A generalized mixed linear model was used for analysis of associations of the candidate gene SNPs with the level of whole blood RNA expression of their respective candidate genes (*CD163*, *CD169*, and *RSG16*) across time points in the coinfection trials, separately for time points pre- and post coinfection. The final model was the same for the pre- and the post coinfection data sets and did not include any two-way interactions of the candidate SNP genotype, WUR genotype, vaccination status,
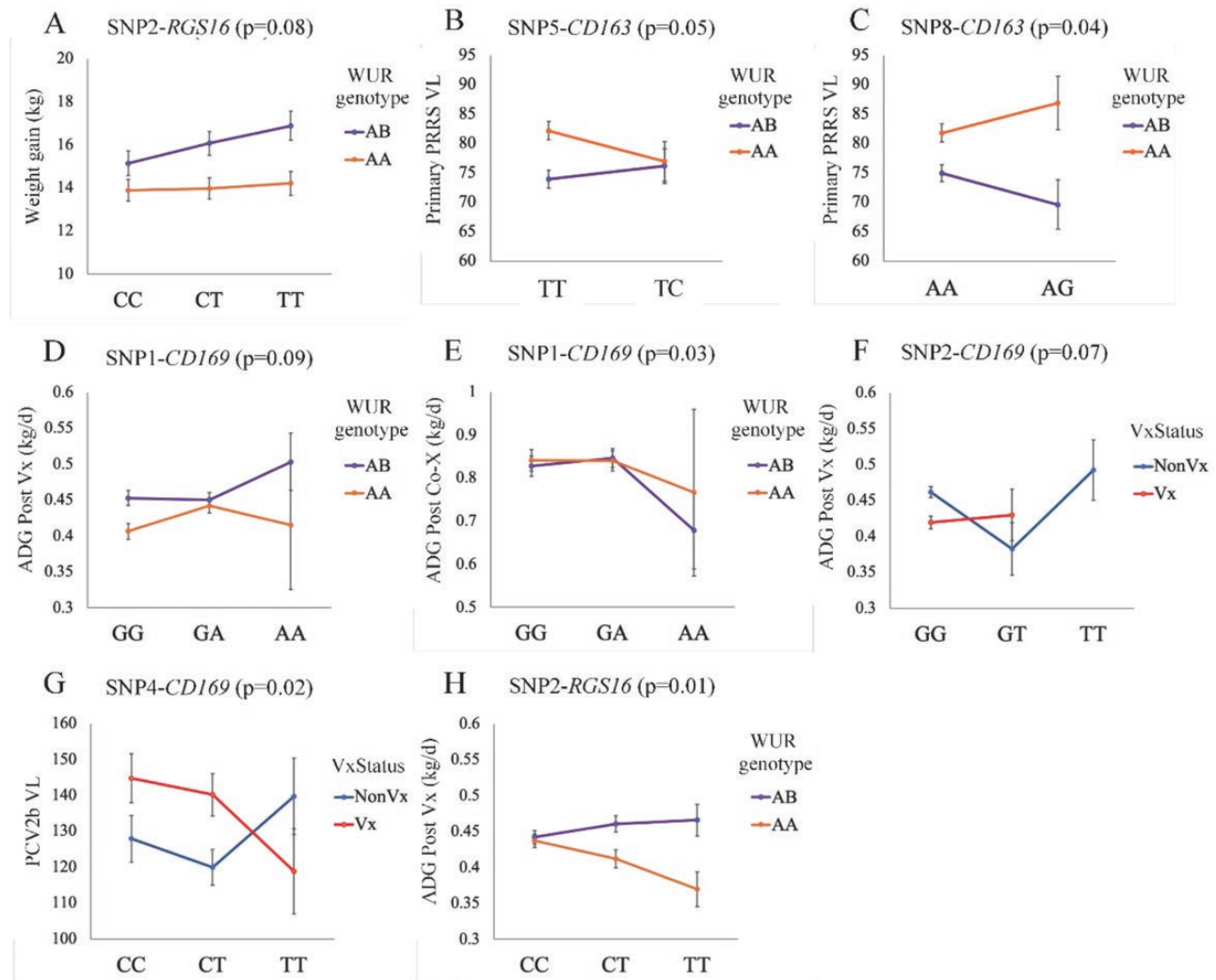
Figure 2. Interaction effects (and SE bars) between genotype of SNPs in candidate genes and WUR genotype or PRRSV vaccination status. Effects of SNP2 in the *RGS16* gene with a significant interaction effect with WUR genotype on weight gain from 0 to 42 days post infection in the PRRSV-only infection trials (A). Effects of SNP 5 (B) and 8 (C) in the *CD163* gene that had significant interaction effects with WUR genotype on vaccination viral load of vaccinated pigs and PRRS viral load post coinfection of non-vaccinated pigs. Effects of SNP 1 in the *CD169* gene with significant (nominal P = 0.09 and 0.03) interaction effects with WUR genotype on growth rate post PRRS vaccination (D) and growth rate post coinfection (E) in the coinfection trials. Effects of SNPs 2 and 4 in the *CD169* gene with significant interaction effects with PRRS vaccination status on growth rate postvaccination (F) and PCV2b viral load (G). Effects of SNP 2 in the *RGS16* gene with significant interaction effects with WUR genotype on growth rate postvaccination (H). Growth rate was calculated as the regression of body weight on dpi using body weight data from –28 to 0 and 0 to 42 days post coinfection for growth rate postvaccination and post coinfection, respectively. Vaccination viral load, PRRS viral load post coinfection, and PCV2b viral load were calculated for each individual as the area under the curve of log-transformed viremia from –28 to 0, 0 to 21, and 0 to 42 days post coinfection, respectively. PRRS, porcine reproductive and respiratory syndrome; PRRSV, PRRS virus; SNP, single nucleotide polymorphism; PCV2b, porcine circovirus 2b.

and dpv or dpi because none were significant ($P > 0.1$). Before coinfection, genotypes at SNPs 7, 8, 15, 16, and 18 in the *CD163* gene were significantly associated with *CD163* expression ($P < 0.1$; Table 5). After coinfection, genotypes at SNPs 5 (in complete LD with SNPs 11, 12, and 13), 15, 16, 18, and 19 in the *CD163* gene were significantly associated with *CD163* expression ($P < 0.1$; Table 5). For all these SNPs, heterozygotes had a greater level of expression than homozygotes for the major gene.

For the *CD169* gene, genotypes at SNPs 1 and 4 were significantly associated with *CD169* expression before coinfection, while genotypes at SNPs 1, 3, and 4 were significantly associated with *CD169* expression after coinfection ($P < 0.1$; Table 5). For SNPs 1 and 4, homozygotes for the major alleles had the highest expression of *CD169*, both before and after coinfection. For SNP 3, homozygotes for the major allele had the

lowest level of expression after coinfection. Finally, for the *RGS16* gene, genotypes at SNPs 1 and 2 were significantly associated with *RGS16* expression before coinfection, while genotypes at SNP 1 were also significantly associated with *RGS16* expression after coinfection but in the opposite direction ($P < 0.1$; Table 5). Homozygotes for the major allele at this SNP had the highest level of expression.

Discussion

This study evaluated and validated the association of SNPs in four candidate genes (*CD163*, *CD169*, *RGS16*, and *TRAF1*) with host response to PRRSV infection in both PRRSV-only and PRRSV/PCV2b coinfection trials. The candidate genes and SNPs were selected based on the known involvement of these genes

Table 3. Estimates (and SE) of the genotype effects at SNPs in the *CD163* gene that interacted with WUR genotype on weight gain and PRRS viral load in PRRSV-only infection trials 1–3

Phenotype	SNP	Alleles[1]	Estimates (SE) of SNP genotype effects for the AA WUR genotype			Estimates (SE) of SNP genotype effects for the AB WUR genotype			Nominal P-value of WUR by SNP interaction
			0[2]	1	2	0	1	2	
Weight gain[3]	5	T/C	−1.7 (0.5)[a]	−1.7 (0.7)[a]	−3.8 (2.3)[a]	0[a]	2.8 (1.1)[b]	–	0.013
	11_12_13	C_G_C/T_A_A	−1.7 (0.5)[a]	−2.2 (0.7)[a]	−3.9 (2.3)[a]	0[a]	2.5 (1.0)[b]	–	0.009
	15	T/C	−1.5 (0.6)[a]	−1.3 (0.6)[a]	−2.8 (1.5)[a]	0[a]	1.1 (0.7)[a]	1.7 (1.9)[a]	0.279
	18	T/C	−1.6 (0.6)[a]	−1.4 (0.6)[a]	−3.9 (1.4)[b]	0[a]	1.0 (0.7)[a]	1.5 (1.7)[a]	0.156
	19	A/G	−1.5 (0.6)[a]	−1.3 (0.6)[a]	−3.6 (1.6)[a]	0[a]	1.2 (0.7)[b]	1.8 (1.9)[ab]	0.162
PRRS viral load[4]	5	T/C	4.9 (0.8)[a]	−0.8 (1.3)[b]	4.0 (4.2)[ab]	0[a]	−6.8 (1.9)[b]	–	0.582
	11_12_13	C_G_C/T_A_A	4.8 (0.8)[a]	−0.3 (1.3)[b]	4.2 (4.2)[ab]	0[a]	−6.3 (1.8)[b]	–	0.557
	15	T/C	4.9 (1.0)[a]	2.5 (1.1)[b]	−1.2 (2.8)[b]	0[a]	−2.5 (1.3)[b]	−3.6 (3.5)[ab]	0.844
	18	T/C	4.8 (1.0)[a]	2.4 (1.1)[b]	3.5 (2.5)[ab]	0[a]	−2.6 (1.4)[b]	−2.8 (3.2)[ab]	0.919
	19	A/G	4.7 (1.0)[a]	2.3 (1.1)[b]	−0.9 (3.0)[ab]	0[a]	−2.8 (1.3)[b]	−3.7 (3.5)[b]	0.875

[1]The second letter indicates the minor allele.
[2]Homozygotes for the major allele; homozygotes for the minor allele not shown because of large SE.
[3]Weight gain, calculated as body weight at day 42 minus body weight at day 0, in kg.
[4]PRRS viral load, calculated as the area under the curve from 0 to 21 dpi.
[a,b]Estimates with different letter superscripts within a row and WUR genotype are significantly different at a nominal $P < 0.1$.
–Indicates few or zero individuals with this genotype.
PRRS, porcine reproductive and respiratory syndrome; PRRSV, PRRS virus.

in host responses to PRRSV and/or PCV2 infection and previous association results. The large sample sizes of the studies used here substantially add to the knowledge of the potential roles of these genes and on SNPs in these genes that can be used to select for improved host response to PRRSV and/or PCV2 infection. Detailed results are discussed in the following sections.

Effects at CD163

Since the *CD163* gene was not in the pig genome build before the 11.1 version, the SNP genotyping that was used in the PHGC trials did not include SNPs in the *CD163* gene. Therefore, we custom genotyped pigs from the PHGC trials for selected SNPs in the *CD163* gene in order to identify associations with PRRS/PCV2b viral load and weight gain under PRRSV-only and coinfection challenges. Several significant associations were identified. As noted in the following, results suggest the influence of one quantitative trait loci (**QTL**) around SNPs 5, 11_12_13, 15, 18, and 19 in the *CD163* gene, with heterozygotes having more favorable host responses. Effects, however, depended on genetic background, that is, genetic source, likely because of differences in LD, and on genotype at the WUR SNP for the *GBP5* gene. There was evidence of another QTL around SNP 7 in the *CD163* gene, for which homozygotes for the major allele had a more favorable host response in the coinfection trials.

Effects at CD163 depend on genetic background

In the PRRSV-only trials, five SNPs in the *CD163* gene were significantly associated with PRRS viral load (Table 1). These SNPs clustered into two groups that were in high LD with each other (Figure 1): SNPs 5 and 11_12_13, and SNPs 15, 18, and 19. These groups either represent two QTL or each group of SNPs picks up part of the same QTL. To investigate this further, we fitted one SNP from each of these two groups simultaneously in the model, along with their interaction, both for all trials and for trials 1–3 only. In this model (results not shown), the effects of SNPs 5 and 11_12_13 were still significant but the effects of SNPs 15, 18, and 19 and their interaction with a SNP from the other group were not significant. This suggests that there may only be one QTL in this region.

For PRRS viral load in the PRRSV-only trials, all significant *CD163* SNPs had significant interaction effects with genetic background (Table 2). Interestingly, homozygotes for the major alleles at SNPs 5 and 11_12_13 were favorable in trials 4–8 but unfavorable in trials 1–3, suggesting that the phase between these SNPs and the QTL is opposite in these two sets of trials. The effects of SNPs 15, 18, and 19 were only significant for trials 1–3 (Table 2). Within the PRRSV-only trials 1–3 and the coinfection trials from the same genetic source, SNPs 15, 18, and 19 in the *CD163* gene were in high LD with each other (Supplementary Figure S1A and S1B). In the coinfection trials, although not significant ($P = 0.39$ and 0.25), the effect of SNPs 5_11_12_13 (in complete LD with each other) on primary PRRS viral load and PRRS viral load post coinfection was also numerically smaller for heterozygotes ($76.6 ± 2.5$ and $68.6 ± 1.8$) than for homozygotes for the major alleles ($78.1 ± 1.2$ and $71.1 ± 0.8$) (Table 6). Our results for SNP 18 in PRRSV-only trials 1–3 confirm results of Lim et al. (2018), with the same direction of effects on PRRS viral load.

Pigs that were heterozygous at SNPs 5_11_12_13, 15, 18, and 19 had a significantly higher level of *CD163* expression after coinfection (Table 5); however, there was no strong significant effect of these SNPs on *CD163* expression before coinfection. Given the role of the CD163 receptor for entry of PRRSV into macrophages (Whitworth et al., 2016), higher expression of
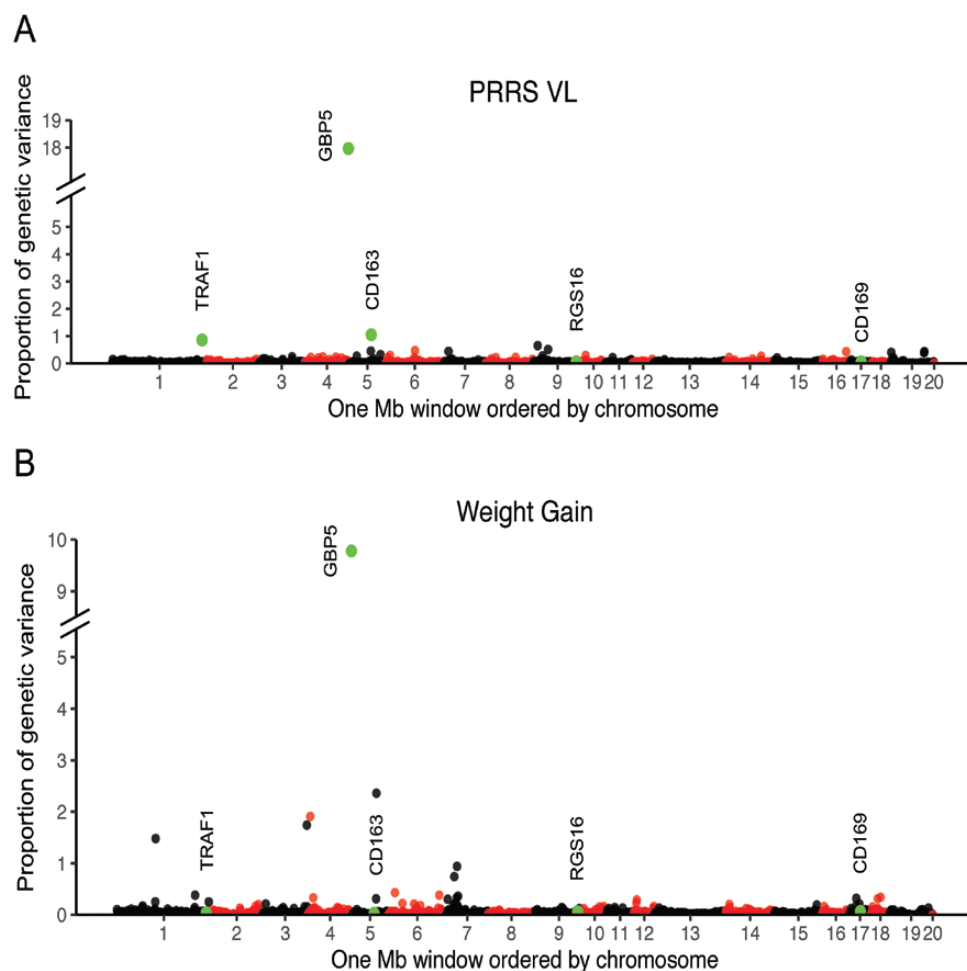
Figure 3. Genome-wide association results for PRRS viral load (A) and weight gain (B) in the PRRSV-only trials using Bayes-B analyses. Results show the percent of genetic variance explained by 1 Mb non-overlapping windows of SNPs across chromosomes. PRRS viral load was calculated as area under the curve of log-transformed viremia from 0 to 21 days post infection. Weight gain was calculated as the difference between body weight at 42 and 0 days post infection. Windows that include genotyped SNPs in the four genes are in green, tagged by the gene name. PRRS, porcine reproductive and respiratory syndrome; PRRSV, PRRS virus; SNP, single nucleotide polymorphism.

CD163 might be expected to be associated with higher PRRS viral load, but the opposite was observed here. This difference might be due to expression of other genes that are needed to complete viral replication in macrophages. We considered confirming the association of SNPs in the *CD163* gene with *CD163* gene expression in a PRRSV-only infection trial (trial 3) that we had RNA-seq data on, as described in Schroyen et al. (2016). However, the minor allele frequency at these SNPs was too small in the 16 pigs evaluated in that study.

Taking all these results together, selecting for the heterozygotes at SNPs 5, 11_12_13, 15, 18, and 19 in the *CD163* gene may improve disease resistance (Table 6). However, this needs further research because we only detected the associations of these SNPs with host response in one genetic source in the PRRSV-only infection trials, while heterozygotes unexpectedly showed higher expression of the *CD163* gene than homozygotes for the major alleles at these SNPs after coinfection.

Genotype at SNP 7 in the *CD163* gene was associated with PCV2b serum viremia in the coinfection trials, as well as with growth rate before and after coinfection (Table 4), and with expression of the *CD163* gene before coinfection (Table 5). Compared with heterozygotes, homozygotes for the major allele (CC) at SNP 7 were favorable in the coinfection trials, with lower

CD163 gene expression before coinfection and lower PCV2b viral load and higher growth rate after coinfection (except for lower growth rate postvaccination, Table 4), although there was no effect on PRRS viral load. Lim et al. (2018) found that pigs with the CC genotype had higher PRRS viremia than CG and GG pigs and a similar trend was observed in our PRRSV-only infection trials (Table 1). In the coinfection trials, SNP 7 had low LD with SNPs 5_11_12_13, 15, 18 (but moderate in the PRRSV-only infection trials), and 19 (Figure 1). Thus, the associations found for SNP 7 may represent another QTL, with effects on PCV2b viral load under coinfection with PRRSV, but more data is needed before strong conclusions can be drawn.

Effects at *CD163* depend on genotype at GBP5

In the PRRSV-only infection trial genome-wide association study, the *GBP5* window (Figure 3), which includes the putative causative major quantitative trait nucleotide for host response to PRRSV infection in the *GBP5* gene (Koltes et al., 2015), explained the largest proportion of genetic variance for both PRRS viral load and weight gain (Figure 3), consistent with our previous results for the same data (Boddicker et al., 2012, 2014a, 2014b; Waide et al. 2017). In the coinfection trials, pigs with the AB genotype at the WUR SNP, which is in high

Table 4. Estimates (SE) of the effect of genotype at SNPs that are associated with host response to PRRS vaccination and PRRSV/PCV2b coinfection

Gene	SNP	Allele[1]	No. of animals with genotype	Minor allele frequency	Trait	Least square means (SE) by SNP genotype			False discovery rate[3]	P-value	Nominal P-value interaction with WUR
						0	1	2[2]			
CD163	5	T/C	362	0.07	Primary PRRS viral load[4]	78.1 (1.2)	76.6 (2.5)	–	1.00	0.39	0.05
CD163	8	A/G	352	0.04		78.4 (1.2)	78.3 (3.5)	–	1.00	0.95	0.04
CD163	7	C/G	349	0.05	PCV2b viral load[5]	129.4 (4.1)[a]	146.9 (9.1)[b]	–	0.35	0.04	–
CD169	1	G/A	322	0.27		133.0 (5.0)[a]	124.9 (5.0)[a]	186.2 (28.6)[b]	0.35	0.03	–
CD163	7	C/G	349	0.05	Growth rate postvaccination[6]	0.44 (0.01)[a]	0.47 (0.02)[b]	–	0.32	0.06	–
CD169	1	G/A	322	0.27		0.43 (0.01)	0.45 (0.01)	0.46 (0.05)	0.64	0.21	0.09
RGS16	2	C/T	334	0.25		0.44 (0.01)	0.44 (0.01)	0.42 (0.02)	0.64	0.56	0.01
CD163	7	C/G	349	0.05	Growth rate post coinfection[7]	0.85 (0.01)	0.78 (0.04)	–	0.38	0.11	
CD169	1	G/A	322	0.27		0.83 (0.02)	0.84 (0.02)	0.72 (0.11)	0.84	0.62	0.03
CD169	3	A/C	346	0.4		0.81 (0.02)[ab]	0.85 (0.01)[a]	0.88 (0.03)[b]	0.38	0.11	–

[1]The second letter indicates the minor allele.
[2]Homozygote of the minor allele.
[3]False discovery rate <0.4.
[4]Primary PRRS viral load: PRRS viral load following first exposure, that is, postvaccination or post coinfection without vaccination.
[5]PCV2b viral load: calculated as the area under the curve from 0 to 42 dpi.
[6]Growth rate postvaccination calculated as the regression of body weight on dpi between PRRS vaccination and PRRSV/PCV2b coinfection.
[7]Growth rate post coinfection calculated as the regression of body weight on dpi post PRRSV/PCV2b coinfection.
[a,b]Estimates with different letter superscripts within a SNP and trial set are significantly different at a nominal P < 0.1.
–Indicates few or zero individuals with this genotype.
PRRS, porcine reproductive and respiratory syndrome; PRRSV, PRRS virus; PCV2b, porcine circovirus 2b.

Table 5. Estimates (SE) of the effects of genotype at SNPs in the *CD163*, *CD169*, and *RSG16* genes that are associated with the expression of their respective genes before and after coinfection with PRRSV and PCV2

| | Gene | SNP | Allele[1] | No. animals with genotype | Minor allele frequency | Least square means (SE) by genotype | | | Nominal P-value[3] |
						0	1	2[2]	
Before coinfection	CD163	5	T/C	362	0.07	3.7 (0.2)[a]	3.7 (0.2)[a]	5.5 (1.0)[b]	0.19
		7	C/G	349	0.05	3.6 (0.3)[a]	4.9 (0.4)[b]	–	0.0001
		8	A/G	352	0.04	3.6 (0.1)[a]	5.6 (0.6)[b]	–	0.0007
		15	T/C	345	0.12	3.7 (0.2)[a]	3.8 (0.2)[a]	5.5 (1.0)[b]	0.04
		16	G/A	352	0.01	3.7 (0.1)[a]	5.3 (0.5)[b]	–	0.0004
		18	T/C	351	0.13	3.6 (0.2)[a]	3.9 (0.2)[a]	5.5 (1.0)[b]	0.05
		19	A/G	337	0.12	3.7 (0.2)[a]	3.7 (0.2)[a]	5.6 (1.0)[b]	0.16
	CD169	1	G/A	322	0.27	2.7 (0.1)[a]	2.3 (0.2)[b]	–	0.01
		2	G/T	357	0.03	2.5 (0.2)	2.8 (0.4)	2.2 (0.7)	0.62
		3	A/C	346	0.40	2.4 (0.2)[a]	2.4 (0.2)[a]	2.9 (0.2)[b]	0.14
		4	C/T	343	0.40	2.8 (0.2)[a]	2.3 (0.2)[b]	2.2 (0.4)[ab]	0.02
	RGS16	1	A/G	320	0.50	0.8 (0.2)[ab]	0.7 (0.2)[b]	1.1 (0.2)[a]	0.099
		2	C/T	334	0.25	0.9 (0.1)[ab]	0.6 (0.2)[a]	1.1 (0.2)[b]	0.07
After coinfection	CD163	5	T/C	362	0.07	3.7 (0.049)[a]	4.1 (0.1)[b]	3.7 (0.4)[ab]	0.0012
		7	C/G	349	0.05	3.8 (0.046)	3.9 (0.1)	–	0.55
		8	A/G	352	0.04	3.8 (0.04)	4.0 (0.2)	–	0.55
		15	T/C	345	0.12	3.7 (0.1)[a]	4.0 (0.1)[b]	3.7 (0.4)[ab]	0.003
		16	G/A	352	0.01	3.8 (0.04)[a]	4.5 (0.3)[b]	–	0.04
		18	T/C	351	0.13	3.7 (0.1)[a]	4.0 (0.1)[b]	3.7 (0.4)[ab]	0.004
		19	A/G	337	0.12	3.7 (0.1)[a]	4.1 (0.1)[b]	3.7 (0.4)[ab]	0.001
	CD169	1	G/A	322	0.27	3.1 (0.1)[a]	2.8 (0.1)[b]	–	0.008
		2	G/T	357	0.03	3.0 (0.1)[a]	2.8 (0.2)[bc]	2.4 (0.4)[c]	0.33
		3	A/C	346	0.40	2.8 (0.1)[a]	3.0 (0.1)[b]	3.3 (0.1)[c]	0.01
		4	C/T	343	0.40	3.1 (0.1)[a]	2.9 (0.1)[b]	2.6 (0.2)[b]	0.02
	RGS16	1	A/G	320	0.50	1.1 (0.1)[a]	0.9 (0.1)[b]	1.0 (0.1)[ab]	0.096
		2	C/T	334	0.25	1.0 (0.1)	0.9 (0.1)	1.0 (0.1)	0.46

[1]The second letter indicates the minor allele.
[2]Homozygote of the minor allele.
[3]Nominal P < 0.1.
[a,b,c]Estimates with different letter superscripts within a SNP are significantly different at a nominal P < 0.05.
–Indicates few or zero individuals with this genotype; PRRSV, PRRS virus.

but not complete LD with the *GBP5* mutation (Jeon et al., 2021), had lower primary PRRS viral load than pigs with the AA genotype at WUR, which is consistent with the findings of Dunkelberger et al. (2017a) for the same data. Pigs with at least one B allele at the WUR SNP are expected to produce a functional GBP5 protein to induce innate immunity for antiviral response (Koltes et al., 2015). Lim et al. (2018) found significant interactions of genotype at WUR with genotype at SNPs 7 and 18 in *CD163* on PRRS viremia and weight gain. This suggests a potential biological interaction between GBP5 and CD163, which was further explored here. For trials 1–3, *CD163* SNPs 5 and 11_12_13 had significant interactions with WUR genotype on weight gain but not on viral load. For weight gain, the effects of these SNPs was significant only for pigs with the AB WUR genotype, with heterozygotes at SNPs 5 and 11_12_13 being favorable (Table 3). For viral load, heterozygotes at these SNPs were favorable (lower viral load) for both WUR genotypes and their interaction was not significant. In the coinfection trials, SNP 5 (in complete LD with SNP 11_12_13) also showed significant interaction effects with WUR genotype for primary PRRS viral load (Figure 2B). However, in these trials, heterozygotes had lower viral load for pigs with the AA WUR genotype but higher viral load for pigs with the AB WUR genotype. For *CD163* gene expression, the interaction between genotype at WUR and genotype at SNP 5_11_12_13 was not significant (results not shown).

Although *CD163* SNP 8 had no significant main effect in either the PRRSV-only or coinfection trials, in the coinfection trials, we identified a significant interaction of genotype at SNP 8 with genotype at WUR on primary PRRS viral load (Figure 2C). For SNP 8, AA pigs had higher primary PRRS viral load than heterozygotes for AB pigs at WUR but lower primary PRRS viral load than heterozygotes for AA pigs at WUR. The result for AA pigs at WUR is consistent with the finding of Ren et al. (2012) that the AA genotype at SNP 8 is associated with a lower relative risk of PRRS incidence. A possible reason why SNP 8 was detected as a main effect by Ren et al. (2012) but only through an interaction with WUR genotype in our studies, may be that most of the pigs in the study of Ren et al. (2012) may have been AA for WUR, while in our coinfection trials, the number of AA and AB pigs at WUR were balanced by design. Our previous studies have noted the low frequency of the B allele at WUR in all commercial pigs investigated (Boddicker et al., 2012). Pigs with the AA genotype at SNP 8 also had lower expression of *CD163* in blood before coinfection than AG pigs (Table 5). In addition, interactions of genotype at SNPs 7 and 8 with genotype at WUR on *CD163* gene expression were suggestive after and before coinfection, respectively (P = 0.11 and 0.13, results not shown). SNP 8 in the *CD163* gene is a non-synonymous c.2592A>G substitution in exon 11, causing a Lys851Arg change. We also identified significant interactions between WUR and *CD163* SNP 15 and 16 on *CD163* gene expression after coinfection (P = 0.05 and 0.06,

Table 6. Comparison of estimates (SE) of the effects of genotype at SNPs in the CD163 gene that were significant in the PRRSV-only infection trials 1–3 with their estimates in the coinfection trials

SNP	Alleles[1]	Least square means (SE) for PRRS viral load[2] by SNP genotype in the PRRSV-only trials				Least square means (SE) for PRRS viral load post coinfection[2] by SNP genotype in the coinfection trials			
		0	1	2[3]	Nominal P-value	0	1	2[3]	Nominal P-value
5	T/C	0[a]	−5.9 (1.0)[b]	−1.0 (4.1)[ab]	1.78×10^{-8}	71.1 (0.8)	68.6 (1.8)	–	0.248
11_12_13	C_G_C/T_A_A	0[a]	−5.4 (1.0)[b]	−0.8 (4.1)[ab]	2.57×10^{-7}				
15	T/C	0[a]	−2.4 (0.7)[b]	−5.6 (2.0)[b]	0.011	71.3 (0.9)	68.4 (1.4)	73.7 (7.4)	0.218
18	T/C	0[a]	−2.4 (0.7)[b]	−1.8 (2.0)[ab]	0.004	71.4 (0.9)	68.5 (1.4)	76.9 (6.9)	0.183
19	A/G	0[a]	−2.5 (0.7)[b]	−4.8 (2.3)[b]	0.001	71.6 (0.9)	68.8 (1.4)	73.8 (7.4)	0.214

[1]The second letter indicates the minor allele.
[2]PRRS viral load, calculated as the area under the curve from 0 to 21 dpi.
[3]Homozygotes for the minor allele.
[a,b]Estimates with different letter superscripts within a SNP are significantly different at a nominal P < 0.1.
–Indicates few or zero individuals with this genotype.
PRRS, porcine reproductive and respiratory syndrome; PRRSV, PRRS virus.

results not shown), and between WUR and *CD163* SNP 1 before coinfection (P = 0.08, results not shown).

In conclusion, although results were not entirely consistent, there is evidence of interaction effects of genotype at SNPs in the *CD163* gene with genotype at *GBP5* on host response to PRRSV infection. The observed interactions may be explained by the biological roles of *GBP5* and *CD163* in macrophages. Macrophages have been broadly classified into classically activated macrophages (M1) and alternatively activated macrophages (M2). In humans, monocyte-derived macrophages can be differentiated into M1, M2a, M2b, and M2c subtypes following stimulation by different cytokines (Fujiwara et al., 2016). M1 can be induced by interferon-γ (IFN-γ) and express proinflammatory molecules, including interleukin-12 (IL-12), CXCL9, CXCL10, CXCL11, nitric oxide, and reactive oxygen species. In humans, GBP5 is a candidate marker that is specifically expressed in human INF-γ-induced M1 macrophages (Fujiwara et al., 2016). However, M2a, M2b, and M2c macrophages can be induced by IL-4, IL-1β, and IL-10, respectively, and M2 cells produce anti-inflammatory molecules, including ornithine, IL-10, CCL17, CCL18, CCL22, and scavenger receptors (Goerdt and Orfanos, 1999; Gordon, 2003; Mosser, 2003; Mantovani et al., 2004; Martinez et al., 2006). CD163 is a marker for M2c macrophages (Fujiwara et al., 2016). If immature macrophages become M1, they will produce GBP5 and, thus, have relatively low CD163 production. Therefore, an immature macrophage in humans can have one of two fates: 1) become M1 expressing GBP5 or 2) become M2c expressing CD163.

In pigs, CD163 surface expression is downregulated by IL-4 and upregulated by IL-10 (Pérez et al., 2008). Moreover, all-trans retinoic acid induces partial alternative activation of porcine M2a through IL-4, and further suppresses CD163 expression (Chen et al., 2019). The level of expression of *CD163* and *GBP5* may depend on the fate of the immature macrophages. When GBP5 is functional, it may have a positive effect on suppressing the receptor function of CD163, especially the interaction effect on PRRS viral load, with genotype at SNPs 5 and 8 in the *CD163* gene and genotype at *GBP5* resulting in antagonistic interactions (Figure 2B and 2C). An interaction between these two genes may also be reflected in the levels of expression of these two genes; using the coinfection trial data, the correlation between count per million levels of expression of the *CD163* and *GBP5* genes was on average negative, although not significant, at time points before coinfection (−0.42±0.52, P = 0.48). However, at time points after coinfection, the correlation was on average positive and significant (0.74±0.34, P = 0.09). The nonsignificant correlation before coinfection might be because mature macrophages are in tissues but not in blood.

Effects at CD169

Although CD169 is not a required receptor for PRRSV infection, unlike CD163, CD169 plays roles in anti-pathogen immune response (reviewed in Neill et al., 2012). Both Wang et al. (2012) and Ren et al. (2012) identified associations of SNPs in the *CD169* gene to be associated with response to PRRSV infection. Several associations were also identified in our study but there was also evidence of interaction effects with genetic background and genotype at WUR.

Main effects at CD169

In the PRRSV/PCV2b coinfection trials, *CD169* SNP 1 was significantly associated with PCV2b viral load, with pigs with the AA genotype showing higher PCV2b viral load than the other two

genotypes (Table 4). Additionally, AG pigs showed significantly lower expression of *CD169* before and after coinfection than GG pigs (Table 5). Because CD169 can enhance CD8 associated immune responses (Neill et al., 2012; van Dinther et al., 2018; Uchil et al., 2019), greater expression of *CD169* gene in GG pigs may alter antiviral immunity. In the PRRSV-only infection trials, pigs with the AA genotype at SNP 1 had higher PRRS viral load than pigs with the other two genotypes at this SNP, while GG pigs tended to have higher weight gain than pigs with the other two genotypes, although these effects were not significant (*P* = 0.21 and 0.24, results not shown). These trends are, however, agree with findings by Wang et al. (2012) that the G allele at SNP 1 is favorable for PRRSV resistance. Based on these results, selection for the G allele at SNP 1 in the *CD169* gene might improve host response to both PRRSV-only infection and PRRSV/PCV2b coinfection.

The non-synonymous SNP 3 (C1654A in exon 6) in the *CD169* gene results in a Leu552Ile substitution in the CD169 protein. In the coinfection trials, SNP 3 in the *CD169* gene had a suggestive association with growth rate post coinfection (*P* = 0.11), with the CC genotype showing higher growth rate post coinfection than the AC genotype (Table 4). Additionally, the AA genotype was associated with significantly lower *CD169* gene expression than the other two genotypes after coinfection (Table 5). However, Ren et al. (2012) showed that pigs with the AA genotype at this SNP had a lower relative risk of PRRSV infection than pigs with the other two genotypes. However, the association of SNP 3 with PRRS viral load was not significant in either the PRRSV-only or coinfection trials. The inconsistency with findings by Ren et al. (2012) might be due to the difference in how host response was determined; Ren et al. (2012) used clinical status, while we used viral load and growth rate. Thus, further research is needed to determine the putative associations of SNP 3 with host response to PRRSV infection.

The non-synonymous SNP 4 in the *CD169* gene is C4175T, causing a change of Ala1392Val. In the coinfection trials, this SNP had a significant interaction effect with vaccination status on PCV2b viral load, which was caused by the negative effect of PRRS vaccination on PCV2b viral load being reversed for pigs with the TT genotype at this SNP (Figure 2G). However, for both vaccinated and non-vaccinated pigs, the CT genotype was suggestively favorable (Figure 2G) in terms of PCV2b viral load. Furthermore, in the coinfection trials, CC pigs at this SNP tended to have higher PRRS viral load post coinfection (*P* = 0.4), and CC pigs also tended to have higher PRRS viral load (*P* = 0.4) and higher weight gain (*P* = 0.6) in the PRRSV-only trials (results not shown). Pigs with the CT genotype at this SNP had significantly lower *CD169* gene expression than CC pigs before and after coinfection (Table 5). Ren et al. (2012) found that CT pigs at this SNP had a lower relative risk of PRRSV infection than the other two genotypes. Therefore, while there is evidence that genotype at this SNP affects PRRS viral load following infection in both the PRRSV-only and coinfection (post coinfection but not primary PRRS viral load) trials, and on PCV2b viral load after coinfection, more validation is needed.

Effects at CD169 depend on genetic background
In the PRRSV-only trials, only SNP 2 was significantly associated with PRRS viral load (Table 1). The non-synonymous SNP 2 (G1640T in exon 6) results in an Arg547Leu substitution in the CD169 protein. Interestingly, SNP 2 also showed a significant interaction with genetic background, with opposite directions of associations between the two homozygotes (Table 2); the association for trials 1–3 was consistent with the finding of Ren

et al. (2012) that the GT genotype for SNP 2 is favorable compared with homozygotes for the major allele (GG). In the coinfection trials, genotype at SNP 2 had a significant interaction with vaccination status on growth rate postvaccination, showing that the GT and GG genotypes had similar growth rate postvaccination among vaccinated pigs (Figure 2F). To summarize, genotype TT at SNP 2 in the *CD169* gene was associated with favorable host response in PRRSV-only trials 1–3 but not in trials 4–8. Thus, the associations for this SNP need further research, including under coinfection.

Effects at CD169 depend on genotype at GBP5
SNP 1 in the *CD169* gene is a non-synonymous c.878A>G SNP in exon 4, causing a change of Arg293His. In the coinfection trials, the significant interactions between genotype at this SNP with WUR genotype on growth rate postvaccination and post coinfection (Figure 2D and 2E) indicate that CD169 may interact with GBP5. However, we did not identify a significant average correlation across time points between the level of expression of the *CD169* and *GBP5* genes before (0.03±0.58, *P* = 0.97) or after (–0.45±0.45, *P* = 0.38) coinfection. We also did not identify significant interactions between WUR genotype and genotype at any of *CD169* SNPs on *CD169* gene expression, either before or after coinfection.

Effects at RGS16
The two SNPs in the *RSG16* gene are located in the 5′ upstream region of the *RGS16* gene (Supplementary Table S1). To investigate this further, the TFBIND software (Tsunoda and Takagi, 1999) was used to search for transcription factor (TF) binding sites by inputting the sequence ±30 bp around the SNP. Supplementary Table S5 shows the predicted TFs that may bind to the sites that include one of the two alleles of the SNPs in the 5′ upstream region of the *RGS16* gene. Thus, a mutation at these sites may prevent or enable the TF to bind to the promoter or enhancer region of the *RGS16* gene, resulting in changes in the level of RGS16 transcription. Interestingly, a P300 binding motif was found only in the sequence that included the T allele at SNP 2 in the *RGS16* gene (Supplementary Table S5). The P300 protein, which is encoded by the *EP300* gene, is a histone acetyltransferase activator of gene expression in several systems, including the inflammatory/immune response (Revilla and Granja, 2009). RGS16 is known to interact directly with ORF3 of PCV2 and may play an important role in translocation of the ORF3 protein of PCV2 into the cell nucleus (Timmusk et al., 2009) and harbor PCV2 proteins in infected cells (Vincent et al., 2003, 2005, 2007). Additionally, the *RGS16* gene is upregulated in immature dendritic cells (Shi et al., 2004), in which PCV2 can persist (Vincent et al., 2003), and *RGS16* inhibits maturation of dendritic cells through TLR9 (Vincent et al., 2005). Therefore, a nonfunctional RGS16 may inhibit PCV2 infection and increase growth rate.

In the coinfection trials, pigs with the AA genotype at SNP 1 in the *RGS16* gene had significantly higher *RGS16* gene expression after coinfection than pigs with the AG genotype (Table 5). However, the associations of SNPs 1 and 2 with PCV2b viral load were not significant (*P* = 0.51 and 0.13, result not shown), although heterozygotes for SNP 2 tended to have lower PCV2b viral load than the other two genotypes. Lim et al. (2017) found that pigs with genotype AG at SNP 1 and genotype TC at SNP 2 had significantly higher PCV2b viral load than pigs with the other two genotypes. The inconsistency of the associations of SNPs in the *RGS16* gene with PCV2 viral load in our coinfection study with those observed by Lim et al. (2017) might be caused

by differences in the nature of the PCV2 infection between the two studies, that is, PRRSV/PCV2b coinfection vs. a natural PCV2-only infection with an unknown strain for Lim et al. (2017). In our PRRSV-only infection trials, pigs with the AA genotype at SNP 1 in the *RGS16* gene tended to have higher PRRS viral load than pigs with the other two genotypes (Table 1) but these differences were not significant. In conclusion, in both the PRRSV-only and the coinfection trials, the G allele at SNP 1 and the CT genotype at SNP 2 in the *RGS16* gene appeared to be favorable, with lower PCV2b viral load compared to the other two genotypes. This suggests that there might be two QTL with different effects around these two SNPs.

Effects at RGS16 *depend on genotype at* GBP5

Interestingly, for both the PRRSV-only infection trials and the PRRSV/PCV2b coinfection trials, SNP 2 showed a significant interaction with WUR genotype on weight gain (Figure 2A) and weight gain postvaccination (Figure 2H), respectively, which suggests that RGS16 may interact with GBP5. Such interactions were found in RAW 264.7 macrophages, in which both single treatment with IFN-β and dual treatment of IFN-β and KDO (2-keto-3-deoxyoctonate, a ligand that binds TLR4) increased *GBP5* gene expression and decreased *RGS16* gene expression (dual treatment only; Krishnan and Choi, 2012). Interestingly, the average correlation between *GBP5* and *RGS16* gene expression was negative across time points before coinfection (0.83±0.32, $P = 0.08$) but not after coinfection (0.33±0.47, $P = 0.53$). Moreover, we did not identify significant interactions between genotype at WUR and genotypes at either of the *RGS16* SNPs on *RGS16* gene expression, either before or after coinfection.

Effects at TRAF1

The *TRAF1* gene is a negative regulator of TNF signaling in T lymphocytes (Tsitsikov et al., 2001). No SNP in *TRAF1* was significantly associated with any trait in either the PRRSV-only or the coinfection trials. This may in part be due to the low MAF at these SNPs: 0.003 in the coinfection trials and 0.10 in the PRRSV-only trials. In the PRRSV-only infection trials, P were 0.53 for PRRS viral load and 0.13 for weight gain. However, the 1 Mb window that contains *TRAF1* did explain 0.9% of the genetic variation for PRRS viral load in the PRRSV-only trials. This window was also identified to be associated with PRRS viral load of NVSL in our previous genome-wide association study of the PRRSV-only trials (Waide et al., 2017), noting that this window was mapped to 292–293 Mb on chromosome 1 in build 10.2 of the swine genome that was used in that study. Of the top ten SNPs in the *TRAF1* window with associations with PRRS viral load, six are located in the *CDK5RAP2* gene (ASGA0007320, ALGA0009926, M1GA0001522, DIAS0000208, H3GA0004641, and ASGA0007309). A previous study has suggested that mutations in the *CDK5RAP2* gene play roles in impaired centrosomal function and mitotic progression with abnormal mitotic spindle orientation in humans (Lizarraga et al., 2010). Loss of CDK5RAP2 showed smaller cerebral organoids containing fewer neural progenitor cells with aberrant cell polarity in humans (Gharbaran and Somenarain, 2019). The SNP DRGA0002443 in this same 1 Mb window is located in the *MEGF9* gene and was associated with PRRS viral load. MEGF9 is a transmembrane protein with multiple EGF-like domains and could play a role in the development and maintenance of the nervous system (Brandt-Bohne et al., 2007). Highly pathogenetic PRRSV infection causes severe neurological symptoms such as shivering and PRRSV has been identified in the brain of PRRSV-infected piglets (Tian et al., 2007). Mutations

in both the *CDK5RAP2* and *MEGF9* genes may cause aberrant nervous system symptoms through the change of their gene expression under PRRSV infection.

Conclusions

We identified and evaluated the associations of natural SNPs in four candidate genes, *CD163*, *CD169*, *TRAF1*, and *RGS16*, with host response to PRRSV and/or PCV2b infection. Several SNPs in the *CD163*, *CD169*, and *RGS16* genes showed significant associations with PRRSV and/or PCV2b viral load, and/or growth rate following PRRSV-only infection or PRRSV/PCV2b coinfection and PRRS vaccination. All the SNPs in the *CD163* and *CD169* genes that were significant in the PRRSV-only infection trials had significant interaction effects with the genetic backgrounds evaluated. In general, we suggest that selection of heterozygotes of SNPs 5, 11_12_13, 15, 18, and 19 in the *CD163* gene, for the G allele at SNP 1 in the *CD169* gene, and for the G allele at SNP 1 in the *RGS16* gene is expected to improve host response to PRRSV and/or PCV2 infection. Additionally, several SNPs in the *CD163*, *CD169*, and *RGS16* genes showed significant interaction effects with genotype at the WUR SNP, which is in almost complete LD with the putative causative mutation for PRRS resistance in the *GBP5* gene. This suggests a biological interaction between *GBP5* and these candidate genes. Additional research is needed to confirm these findings and understand the mechanisms behind the effects of these SNPs on PRRS and/or PCV2 viral load, and/or growth rate following (co-)infection.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

Acknowledgments

Conflict of interest statement

The authors declare they have no competing interests.

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