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Genomewide association of piglet responses to infection with one of two porcine reproductive and respiratory syndrome virus isolates[1]

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ABSTRACT: Porcine reproductive and respiratory syndrome (PRRS) is a devastating disease in the swine industry. Identification of host genetic factors that enable selection for improved performance during PRRS virus (PRRSV) infection would reduce the impact of this disease on animal welfare and production efficiency. We conducted genomewide association study (GWAS) analyses of data from 13 trials of approximately 200 commercial crossbred nursery-age piglets that were experimentally infected with 1 of 2 type 2 isolates of PRRSV (NVSL 97-7985 [NVSL] and KS2006-72109 [KS06]). Phenotypes analyzed were viral load (VL) in blood during the first 21 d after infection (dpi) and weight gain (WG) from 0 to 42 dpi. We accounted for the previously identified QTL in the *GBP5* region on SSC4 in our models to increase power to identify additional regions. Many regions identified by single-SNP analyses were not identified using Bayes-B, but both analyses identified the same regions on SSC3 and SSC5 to be associated with VL in the KS06 trials and on SSC6 in the NVSL trials ($P < 5 \times 10^{-6}$); for WG, regions on SSC5 and SSC17 were associated in the NVSL trials ($P < 3 \times 10^{-5}$). No regions were identified with either method for WG in the KS06 trials. Except for the *GBP5* region on SSC4, which was associated with VL for both isolates (but only with WG for NVSL), identified regions did not overlap between the 2 PRRSV isolate data sets, despite high estimates of the genetic correlation between isolates for traits based on these data. We also identified genomic regions whose associations with VL or WG interacted with either PRRSV isolate or with genotype at the SSC4 QTL. Gene ontology (GO) annotation terms for genes located near moderately associated SNP ($P < 0.003$) were enriched for multiple immunologically (VL) and metabolism- (WG) related GO terms. The biological relevance of these regions suggests that, although it may increase the number of false positives, the use of single-SNP analyses and a relaxed threshold also increased the identification of true positives. In conclusion, although only the SSC4 QTL was associated with response to both PRRSV isolates, genes near associated SNP were enriched for the same GO terms across PRRSV isolates, suggesting that host responses to these 2 isolates are affected by the actions of many genes that function together in similar biological processes.

Key words: function analysis genomewide association study, genetics, genomewide association study, genomics, pig, porcine reproductive and respiratory syndrome virus

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INTRODUCTION

The PRRS Host Genetics Consortium (**PHGC**) investigates host genetic control of response to porcine reproductive and respiratory syndrome virus (**PRRSV**) infection (Lunney et al., 2011). Analysis of PHGC infection trials showed that weight gain (**WG**) and viral load (**VL**) responses to experimental infection with the NVSL 97-7985 (**NVSL**; Osorio et al., 2002) PRRSV isolate were moderately heritable (approximately 0.30) and controlled by a major QTL on SSC4 (Boddicker et al., 2012). One SNP in this QTL, WUR10000125 (**WUR**), captured 99.3% of the genetic variance (**GV**) at the QTL and was validated in independent NVSL trials (Boddicker et al., 2014a,b).

The diversity of PRRSV in the field emphasizes the importance of research involving multiple virus isolates (Murtaugh et al., 2010). To expand the findings from the NVSL trials, the KS2006-72109 (**KS06**) isolate, which shares 89% Open Reading Frame 5 sequence similarity with NVSL, was used in additional infection trials. Hess et al. (2016) showed that the SSC4 QTL also affected VL for the KS06 isolate but not WG. Hess et al. (2016) also estimated high genetic correlations for VL (0.86 ± 0.19) and WG (0.86 ± 0.27) between the 2 PRRSV isolates, which led us to hypothesize that the same genomic regions control host response to both PRRSV isolates.

Genomic evaluation of complex traits, such as host response to PRRSV, may benefit from the inclusion of biological information, such as gene ontology (**GO**) annotation (Fortes et al., 2011; Serão et al., 2013). Therefore, the objectives of this study were to use genomewide association analyses to identify genomic regions associated with response to PRRSV and to use functional analyses to support these findings. Genomic regions that are associated with host response to both PRRSV isolates may prove useful in industry applications and may also control response to other pathogens. Regions associated with response to only 1 PRRSV isolate may give insight into isolate-specific pathogenicity. Enrichment of relevant GO terms in genes near associated SNP adds functional information to annotate and support the identified statistical associations.

MATERIALS AND METHODS

All experimental protocols used in this study were approved by the Kansas State University (**KSU**) Institutional Animal Care and Use Committee.

Study Design

Lunney et al. (2011) provided a detailed description of the design of the PHGC trials that were used in this study. Briefly, a total of 15 trials of approximately 200 commercial crossbred piglets each were sent to KSU at weaning, given 1 wk to acclimate, and then inoculated intramuscularly and intranasally with 10^5 tissue culture infectious dose (**TCID50**) of either the NVSL (Osorio et al., 2002) or the KS06 PRRSV isolate. In total, 2,289 commercial crossbred piglets from 8 genetic backgrounds were used. Each trial consisted of pigs from only 1 genetic background. A more detailed description of the pigs used in each trial is presented in Table 1. Trial 9 (Dunkelberger et al., 2015) involved pigs from the Iowa State University residual feed intake selection lines (Cai et al., 2008) and was excluded from these analyses. Trial 13 was also excluded, because piglets from this trial had much lower and more variable viremia profiles compared with the other KS06 trials (data not shown).

For each trial, blood samples were collected at 0, 4, 7, 11, and 14 d after infection (**dpi**) and then weekly until completion of the trial at 42 dpi. Individual weights were observed weekly throughout the experimental period. At 42 dpi, piglets were euthanized and ear tissue was collected for genomic DNA extraction, which was sent to GeneSeek, Inc. (Lincoln, NE; trials 1–10), or Delta Genomics (Edmonton, AB, Canada; trials 11–15) and genotyped using the Illumina Porcine SNP60 BeadChip (Ramos et al., 2009). Genotypes with a gene call score lower than 0.5 were set to missing. After removal of SNP with a minor allele frequency less than 0.01 across all trials and with a genotyping call rate less than 0.80, 52,386 SNP remained for analysis, with an overall genotype rate of 99.2%.

Phenotypes

The 2 phenotypes analyzed in this study, VL and WG, were described by Boddicker et al. (2012). Briefly, the amount of PRRSV RNA in blood samples was quantified using quantitative PCR and reported as the $\log_{10}$ of PRRSV RNA copies relative to a standard curve. Viral load was calculated as the area under the curve of $\log_{10}$ viremia up to and including 21 dpi, avoiding the lowly heritable rebound phase of PRRSV infection (after 28 dpi; Boddicker et al., 2012). Viral load was chosen for analysis in this study because it represents an overall measure of a piglet's immune response to PRRSV infection and was shown to be more heritable than individual curve parameters (Hess et al., 2016). Viral load also had a high genetic correlation between the 2 PRRSV isolates studied (Hess et al.,

Table 1. General information on each porcine reproductive and respiratory syndrome infection trial

Trial	No. of VL[1]	No. of WG[2]	Sex[3] Male	Sex[3] Female	Breed[4]	Initial age (SD), d	Initial weight (SD), kg	VL[1] (SD)	WG[2] (SD), kg	PRRSV[5] isolate
1–3	504	487	504	0	LW × LR	26.6 (0.9)	6.4 (1.3)	108.3 (8.1)	12.1 (4.4)	NVSL
4	192	190	85	107	Duroc × LW/LR	26.5 (2.0)	7.9 (1.1)	113.2 (6.3)	15.9 (4.0)	
5	184	183	83	102	Duroc × LR/LW	26.9 (0.5)	7.3 (0.8)	101.4 (7.2)	19.1 (2.9)	
6	123	106	123	0	LR × LR	26.0 (0.9)	7.8 (1.5)	109.6 (8.0)	14.8 (5.6)	
7	189	189	105	84	Pietrain × LW/LR	27.9 (2.3)	8.4 (1.5)	104.5 (6.2)	14.5 (3.2)	
8	188	182	91	97	Duroc × Y/LR	24.9 (4.9)	7.5 (1.7)	107.9 (6.6)	10.2 (4.6)	
15	171	165	176	0	LR × LW	26.0 (1.5)	6.9 (1.3)	107.6 (10.9)	19.1 (4.0)	
NVSL	1,551	1,502	1,167	390		26.6 (2.4)	7.2 (1.4)	107.0 (8.4)	14.9 (5.0)	
10	174	179	185	0	LR × LW	25.5 (1.6)	6.5 (1.4)	93.9 (6.7)	19.1 (4.2)	KS06
11	170	178	185	0	LW × LR	27.8 (1.8)	7.5 (1.1)	100.4 (6.4)	18.6 (4.4)	
12	171	170	181	0	LR × LW	27.2 (1.8)	6.5 (1.4)	104.7 (6.3)	19.0 (4.1)	
14	180	171	90	85	Duroc × LR/LW	26.1 (1.6)	6.7 (1.0)	98.6 (7.7)	21.3 (4.1)	
KS06	695	641	641	85		26.7 (1.9)	6.8 (1.3)	99.4 (7.8)	19.5 (4.3)	
Total	2,246	2,200	1,623	475	–	26.6 (2.4)	7.2 (1.4)	104.6 (8.9)	16.4 (5.2)	

[1]Viral load (VL) is calculated as the area under the curve of log-transformed viremia in serum from 0 to 21 d after infection (dpi).

[2]Weight gain (WG) from 0 to 42 dpi

[3]Number of animals for each sex include animals for which at least 1 trait was observed. Trial 14 had 5 animals for which sex was not recorded.

[4]LW = Large White; LR = Landrace; Y = Yorkshire.

[5]PRRSV = porcine reproductive and respiratory syndrome virus; NVSL = NVSL 97-7985; KS06 = KS2006-72109.

2016). Weight gain was calculated as the difference between BW at 42 and 0 dpi.

Genomewide Association Studies

Associations of SNP genotypes with each trait were assessed using Bayesian variable selection and single-SNP methods, as described in the following. To increase power to identify genomic regions beyond the SSC4 QTL, genotype at the WUR SNP was fitted as a fixed effect in our models and the WUR SNP and all SNP within 2.5 Mb on either side of the WUR SNP were removed from the genomewide association study (**GWAS**).

Single-SNP Analyses. The following linear mixed model was used in ASReml 4 (Gilmour et al., 2015) to separately associate the genotypes at each SNP *i* with phenotypes:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_i\mathbf{g}_i + \mathbf{Wp} + \mathbf{Sl} + \mathbf{Vu} + \mathbf{e},$$

in which **y** = vector of phenotypic observations; **X** = design matrix associating phenotypes with fixed effects; **b** = vector of fixed effects of the interaction of parity and trial, covariates of initial age and weight, and genotype at the WUR SNP; $\mathbf{Z}_i$ = design matrix for the fixed effect of genotype for SNP *i*; $\mathbf{g}_i$ = vector of genotype class effects for SNP *i*; **W** = incidence matrix associating phenotypes with pen within trial; **p** = vector of random effects of pen within trial, assumed to be normally and independently distributed (**NID**) $\sim(\mathbf{0}, \sigma_p^2)$; **S** = incidence matrix associating phenotypes with litter effects; **l** = vector of random effects of litter, assumed to be NID $\sim(\mathbf{0}, \mathbf{I}\sigma_l^2)$; **V** = incidence matrix associating phenotypes with random animal polygenic effects; **u** = vector of random animal polygenic effects, assumed to be normally distributed (**ND**) $\sim(\mathbf{0}, \mathbf{A}\sigma_u^2)$, in which **A** is the additive genetic relationship matrix; and **e** = vector of residual errors assumed to be identically and independently distributed (**i.i.d.**) $\sim N(\mathbf{0}, \mathbf{I}\sigma_e^2)$. A 1-generation pedigree of 4,807 animals, which included the 2,288 animals infected with the PRRSV and their parents, was used to construct **A**. The interaction of parity and trial accounts for differences in the genetic background of pigs, which was confounded with trial. Results are presented as the $-\log_{10}(P)$ of the combined additive and dominance effects at each SNP. Interactions of genotype at each SNP with PRRSV isolate and with WUR genotype were also tested on the combined data set, using the same model described above but including virus isolate and its interaction with SNP genotype or the interaction of SNP genotype with WUR genotype as additional fixed effects.

Bayesian Analyses. All SNP genotypes were simultaneously fitted as random effects using the Bayes-B method (Habier et al., 2011) as implemented in GenSel version 4.90 (Fernando and Garrick, 2008). Missing genotypes were replaced with the mean genotype code for that individual's genetic background for that SNP. We used a modified form of the mixed model used in Zeng et al. (2013) to estimate associations of SNP with each phenotype:

$$\mathbf{y} = \mathbf{Xb} + \sum_{i=1}^{k} \mathbf{z}_{ai} a_i \ddot{a}_{ai} + \sum_{i-1}^{k} \mathbf{z}_{di} d_i \ddot{a}_{di} + \mathbf{e},$$

in which **y** and **X** are as described above; **b** = vector of fixed effects of sex, interactions of pen and parity with trial, genotype at the WUR SNP, and covariates of initial age and weight; $\mathbf{z}_{ai}$ = vector of the additive genotype covariates coded as −10, 0, and 10 for the AA, AB, and BB genotypes, respectively, for SNP *i*; a_i = additive effect for SNP *i*; δ_{ai} = indicator for whether the additive effect of SNP *i* was included (δ_{ai} = 1) or excluded (δ_{ai} = 0) from the model for a given iteration of the Markov chain Monte Carlo (**MCMC**); $\mathbf{z}_{di}$ = vector of the dominance genotype covariates coded as 10, 0, and 10 for the AA, AB, and BB genotypes, respectively, for SNP *i*; d_i = dominance effect for SNP *i*; δ_{di} = indicator for whether the dominance effect of SNP *i* was included (δ_{di} = 1) or excluded (δ_{di} = 0) from the model for a given iteration of the MCMC; and **e** = vector of residual errors assumed to be i.i.d. ~N(**0**, $\mathbf{I}\sigma_e^2$). GenSel version 4.90 (Fernando and Garrick, 2008) does not allow for random effects other than SNP to be fitted in the model; therefore, litter was not included as an effect in the Bayes-B model. The prior probability that a given SNP effect was excluded from the model (δ_{ai} = 0 and δ_{di} = 0) was set equal to π = 0.99, fitting approximately 1% or 524 additive effects and 524 dominance effects in each of 51,000 iterations of the MCMC chain, with the first 1,000 iterations designated as burn-in. Results are presented as the percent of GV explained by the sum of additive and dominance effects of SNP within nonoverlapping 1-Mb windows (Wolc et al., 2015) of the genome based on *Sus scrofa* genome build 10.2 (http://www.ensembl.org/Sus_scrofa/Info/Index?db=core; accessed August 6, 2015). Windows that explained more than 1% GV were further discussed. An alternative method of evaluating Bayes-B results, based on the proportion of iterations in which a window explained greater than average percent GV, identified the same associated regions.

Multiple Test Correction. The number of independent tests in the single-SNP analyses was used to set significance thresholds based on Bonferroni correction. The number of independent tests was based on the number of principal components (**PC**) required to capture 99.5% of the variance among SNP genotypes, following Gao et al. (2008). Genotype data from each PRRSV isolate data set were split between chromosomes and then further divided to create chromosomal segments containing a number of SNP equal to half the number of animals in the respective data set. The PC analysis was conducted using the *princomp* function in R version 3.1.3 (R Core Team, 2015) and used to determine the number of PC required to explain 99.5% of the variance of SNP genotypes in each chromosomal segment. The cumulative number of PC across all chromosome segments (n) was considered to be the number of independent tests and used to designate Bonferroni corrected *P*-value thresholds to determine significance of associations in the single-SNP analyses. Principal components analysis results by chromosome are presented in Supplemental Table S1 (see the online version of the article at http://journalofanimalscience.org). The modified Bonferroni correction following Mantel (1980) was also used to derive an alternative 5% genomewide significance threshold as $0.05/(n)^{1/2} = 0.0002$.

Further Analyses of Specific Genomic Regions. Genes located within 500, 250, or 100 kb, based on *Sus scrofa* genome build 10.2, of SNP with *P*-values of <0.001, <0.003, or <0.01, as determined by single-SNP analyses, were compiled into gene lists to perform functional analyses. Genes around the WUR SNP on SSC4 were added to these lists. Resulting gene lists were analyzed for enrichment of GO annotation terms using PANTHER software (Mi et al., 2012). Both the complete GO terms and a "slim" set of terms in both the biological process and molecular function components of the GO were used for annotation enrichment analyses. The slim approach uses a limited set of GO terms that provides broad coverage of the GO annotation (Mi et al., 2012). PANTHER GO slim is a selected subset of the complete gene ontologies, which gives an overview of the ontologies and removes very specific terms. Significance of overrepresentation of GO terms in a gene list was tested using the binomial distribution function (Cho and Campbell, 2000). Genes within 500, 250, or 100 kb of all 52,386 SNP used in the GWAS analyses were used as background for the selected SNP lists for each respective window size. Resulting *P*-values for enrichment of genes with a given GO term were Bonferroni corrected by multiplying the *P*-value for each term within a category by the total number of terms in the category (Sham and Purcell, 2014): 257 for biological process slim (**BPS**), 223 for molecular function slim (**MFS**), and 177 for pathways.

The 10 most overrepresented GO terms for each gene list (500 kb surrounding SNP with $P < 0.001$, 500 kb surrounding SNP with $P < 0.003$, etc.) were compared with determine the significance threshold and window size that resulted in gene lists that were most overrepresented for functionally relevant BPS GO terms or that were found to be enriched for similar phenotypes analyzed in other studies (de Oliveira et al., 2014; Li et al., 2015; Schroyen et al., 2015). Results from these analyses will be presented. The highest en-

riched BPS GO terms in lists of genes within 250 kb of SNP with $P < 0.003$ (up to 10 per list) were recorded.

RESULTS

In total, 2,288 commercial crossbred piglets from 8 genetic backgrounds were infected with 1 of 2 PRRSV isolates. Piglets averaged 26.6 (±2.4) d of age and weighed 7.2 (±1.4) kg at the time of infection. Of this total, 1,557 piglets were infected with NVSL, with an average weight of 7.3 (±1.4) kg and 26.6 (±2.6) d of age at inoculation. Viral load in the NVSL trials was, on average, 107.0 (±8.4) units and average WG was 14.9 (±5.0) kg. The 731 piglets infected with KS06 weighed, on average, 6.8 (±1.3) kg and averaged 26.7 (±1.9) d of age at the time of inoculation. Compared with the NVSL trials, VL in the KS06 trials was lower, at an average of 104.6 (±8.9) units, and WG was higher, on average 19.5 (±4.3) kg. A more detailed description of the animals used in each trial is presented in Table 1 and a comprehensive analysis of the effects of isolate and WUR genotype and estimates of quantitative genetic parameters of these same data are in Hess et al. (2016).

Heritabilities

Using Bayes-B, the 52,386 SNP together explained 53 and 48% of the phenotypic variance for VL in the KS06 and NVSL data, respectively, and 37 and 39%, respectively, of the phenotypic variance for WG in these respective data sets. When genotype at the WUR SNP was fitted as a fixed effect and SNP within 2.5 Mb on either side of the WUR SNP were excluded, marker-based heritabilities decreased 3.5%, on average. After excluding the SSC4 QTL region, the top 50, 22, 62, and 26 1-Mb windows explained 10% of the GV in the KS06 VL, NVSL VL, KS06 WG, and NVSL WG data sets, respectively. The 1-Mb window with the SSC4 QTL explained 8.2, 14.4, 0.05, and 10.4% of the GV for these respective data sets when genotype at the WUR SNP was included as a random rather than as a fixed effect in Bayes-B.

Table 2 shows estimates of heritability and litter effects from the pedigree-based ASReml analyses of WG and VL in the 2 isolate data sets, using the same model as used for single-SNP GWAS. Pedigree based heritability estimates were moderate and slightly higher for VL than for WG for both PRRSV isolates, averaging approximately 0.36 and approximately 0.27, respectively. When genotype at the WUR SNP was not included as a fixed effect in the model, heritability estimates increased slightly, from 0.38 to 0.40, 0.33 to 0.34, 0.24 to 0.25, and 0.28 to 0.30 for KS06 VL, NVSL VL, KS06 WG, and NVSL WG, respectively. The proportion of phenotypic variance explained by litter effects was close to 0 for WG and for VL in the KS06 data but larger for VL in the NVSL data, approximately 0.19 (Table 2).

Genomewide Association Studies

Principal components analysis determined that the number of PC required to explain 99.5% of the variation in SNP genotypes was 20,492, 21,658, and 22,708 for the KS06, NVSL, and combined data sets, respectively (Supplemental Table S1; see the online version of the article at http://journalofanimalscience.org). The resulting adjusted 5% genomewide statistical significance thresholds for the single-SNP analyses were 2.4×10^{-6}, 2.3×10^{-6}, and 2.2×10^{-6} for the KS06, NVSL, and combined data sets, respectively.

Manhattan plots of the Bayesian and single-SNP GWAS results for VL are shown in Fig. 1, and information on the top genomic regions identified by each method is presented in Table 3. Genotype at the WUR SNP was fitted as a fixed effect in all models, which explains the absence of peaks on SSC4. To compare results from the 2 GWAS methods, we plotted the largest $-\log_{10}(P)$ for each 1-Mb window from the single-SNP analysis against the percent GV explained by that window using the Bayesian method (Supplemental Fig. S1; see the online version of the article at http://journalofanimalscience.org). These comparison plots showed that several of the most significantly associated windows were identified by both methods but, overall, more regions were shown to be moderately associated based on the single-SNP method.

For VL in the KS06 data, Bayes-B and single-SNP analyses identified the 57-Mb window of SSC3 (3_57) as the most strongly associated genomic region (apart from WUR), explaining 1.2% GV in the Bayes-B analysis and having a P-value of 5×10^{-6} in the single-SNP analysis. Windows 7_35 and 7_30 explained the second and third highest percent GV in Bayes-B, 0.82 and 0.32%, respectively; these regions were also associated with VL in the KS06 data in single-SNP analysis ($P = 9 \times 10^{-6}$; Table 3). If these 2 windows on SSC7 are assumed to be associated with a single QTL and their GV is summed along with the windows between 7_30 and 7_35, this 6-Mb QTL explained 1.7% GV. Single-SNP analysis also identified associations in the 9_80, 11_28, 12_26, 14_17, and 15_84 regions ($P < 2.2 \times 10^{-5}$), although none of these reached adjusted genomewide significance; these regions explained between 0.03 and 0.13% GV in the Bayes-B analysis.

Table 2. Estimates (SE) of heritability and litter effects obtained by ASReml[1] analyses and of heritability obtained by Bayes-B analyses for viral load and weight gain in response to infection with the KS2006-72109 (KS06) and NVSL 97-7985 (NVSL) porcine reproductive and respiratory syndrome virus isolates

	Viral load			Weight gain		
	Bayes-B	ASReml		Bayes-B	ASReml	
WUR genotype[2]	Heritability	Heritability	Litter	Heritability	Heritability	Litter
KS06						
Included	0.50	0.38 (0.09)	0.0 (0.0)	0.37	0.24 (0.12)	0.06 (0.05)
Excluded	0.53	0.40 (0.09)	0.0 (0.0)	0.37	0.25 (0.12)	0.06 (0.05)
NVSL						
Included	0.42	0.33 (0.1)	0.18 (0.04)	0.34	0.28 (0.08)	0.04 (0.03)
Excluded	0.48	0.34 (0.1)	0.19 (0.04)	0.39	0.30 (0.08)	0.04 (0.03)

[1]Gilmour et al., 2015.

[2]Information on whether the fixed effect of the WUR10000125 (WUR) genotype was included in the model or not (excluded) when estimating genetic parameters.

For VL in the NVSL data, the 2 most significantly associated regions, 6_79 and 6_80, were identified by both Bayes-B (1% GV; Fig. 1A) and single-SNP analyses ($P < 1.6 \times 10^{-7}$; Fig. 1B); other regions were not consistently identified by both GWAS methods. The 2-Mb window from 6_79 to 6_80 explained 2% GV when considered as a single QTL. A third region, on 6_67, was identified only by single-SNP analysis ($P = 1.6 \times 10^{-7}$; Fig. 1B). Results from Bayesian analysis of VL in the NVSL data also showed a region on 1_292 that explained 1.8% GV (Fig. 1A). Single-SNP analysis also identified a genomic region on SSC1 at 247 Mb to be associated with VL in the NVSL data ($P = 2.5 \times 10^{-7}$; Fig. 1B), 45 Mb upstream of the region identified by Bayes-B.

Figure 2 shows Manhattan plots of the Bayesian and single-SNP GWAS for WG; information on the top regions is presented in Table 4. For WG, there were no regions that were identified by both the Bayes-B and single-SNP analyses in the KS06 data. In fact, Bayes-B results showed no regions that explained more than 1% GV (Fig. 2A) and no SNP reached genomewide significance in the single-SNP results (Fig. 2B). The 2 most significantly associated regions from single-SNP analysis were 13_59 and 10_52 ($P = 1.5 \times 10^{-5}$), which explained 0.4 and 0.06% GV, respectively, in the Bayes-B analysis. The most significant region identified by Bayes-B was at 7_113, with 0.9% GV; this region was not found to be strongly associated in the single-SNP results ($P = 0.02$).

For WG in the NVSL data, Fig. 2A shows that windows on 5_68 and 7_35 explained more than 1% GV in the Bayes-B analysis, but these regions were not strongly associated in the single-SNP analysis ($P > 0.005$; Fig. 2B). The most significant single-SNP association was at 17_22 ($P = 6.5 \times 10^{-6}$); this window explained 0.8% GV in the Bayes-B analysis. Window 5_71 was shown to be moderately associated with WG in the NVSL data based on both Bayes-B (0.6% GV) and single-SNP methods ($P = 3.3 \times 10^{-5}$). Considering the 2 windows on SSC5 to be associated with a single QTL, the resulting 4-Mb window from 5_68 to 5_71 explained 2% GV.

There was no overlap of significantly associated regions between the 2 PRRSV isolates analyzed in this study. Supplemental Fig. S2A and S2B (see the online version of the article at http://journalofanimalscience.org) show the maximum $-\log_{10}(P)$ from single-SNP analysis for each 1-Mb window for VL and WG, respectively, in the NVSL data versus the KS06 data. Maximum *P*-values were corrected for the number of SNP in that window. For VL, the most significant regions in the NVSL data had very weak associations in the KS06 data, and vice versa. Window 9_80 was associated with WG in both isolates but did not reach genomewide significance in either data set.

Joint Genomewide Association Study

To further explore similarities and differences of genomic associations in response to the 2 isolates, we used single-SNP analysis to assess the main effect and the interaction of SNP genotypes with PRRSV isolate in the combined data set (Fig. 3A and 3B). Information on regions with the strongest main and interaction effects is presented in Table 5. Genomic regions with the most significant main effects for VL in the combined data set were at 5_70, 6_79, 15_115, 1_205, 6_80, and 9_29 (Fig. 3A). Genomic regions with the most significant interaction effects with PRRSV isolate for VL were at 14_16, 7_33, 7_39, and 12_32 (Fig. 3A). The 12_32 and 14_16 regions were found to be associated with VL in the KS06 data ($P = 0.0001$ and 0.24% GV and $P = 5 \times 10^{-5}$ and 0.31% GV, respectively) but not in the NVSL data ($P = 0.01$ and 0.11% GV and $P = 0.01$ and 0.11% GV, respectively). None of these

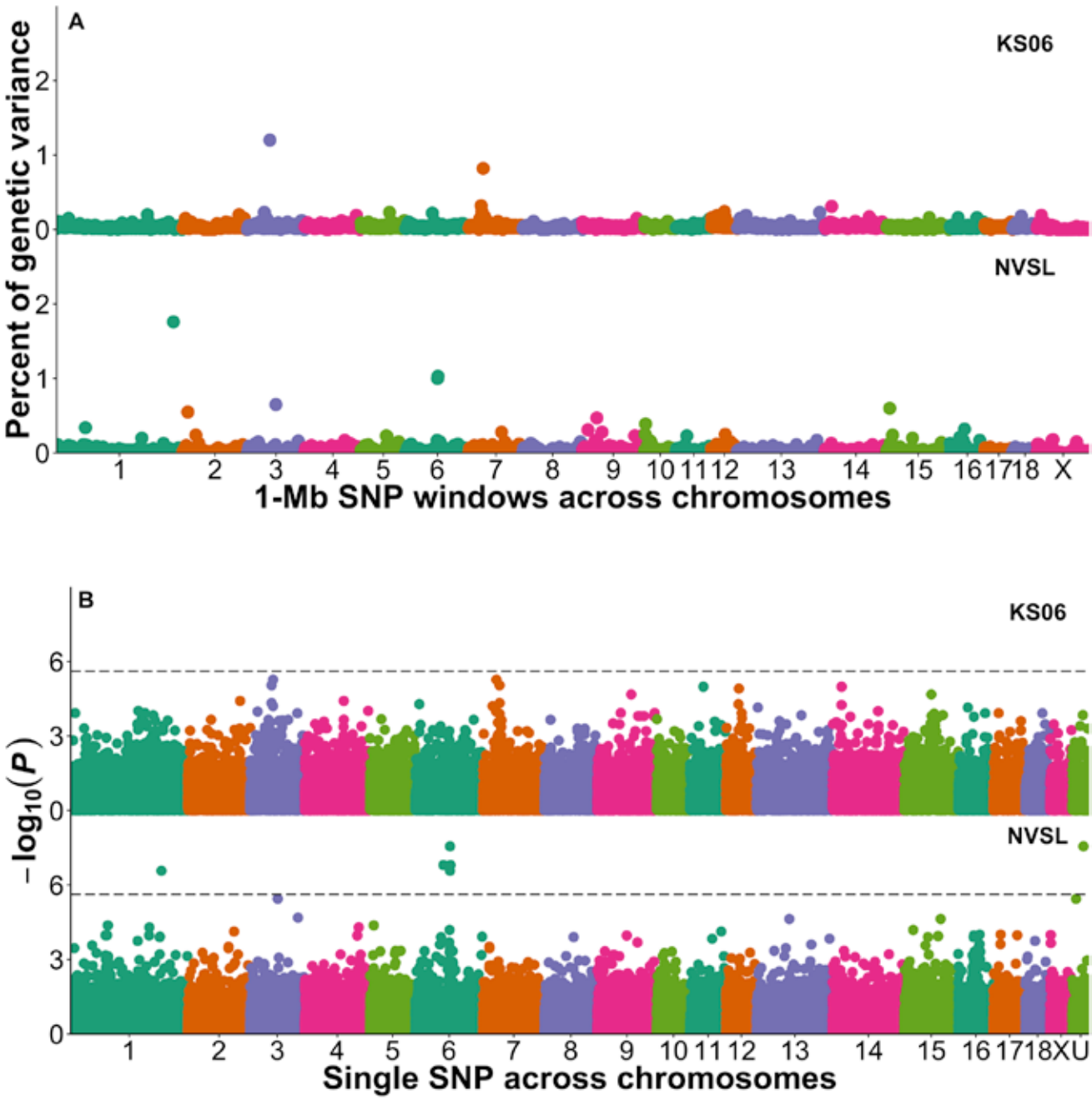

Figure 1. Genomewide association results for viral load using single-SNP and Bayes-B analyses. (A) Bayes-B results show the percent of genetic variance explained by 1-Mb nonoverlapping windows of SNP across chromosomes for KS2006-72109 (KS06; top) and NVSL 97-7985 (NVSL; bottom). (B) Single-SNP analysis results show the $-\log_{10}(P)$ of ordered SNP across chromosomes and for unmapped (U) SNP for KS06 (top) and NVSL (bottom). Dashed lines indicate genomewide significance at a modified Bonferroni corrected *P*-value of 0.05. Results exclude the WUR10000125 region at the end of chromosome 4.

regions reached genomewide significance for interaction with PRRSV isolate after Bonferroni correction.

Figure 3B shows the Manhattan plot for the interaction of genotype at each SNP with PRRSV isolate for WG. Genomic regions with the most significant main effects for WG in the combined data set were at 5_68, 18_21, 5_71, 1_238, and 7_36 (Fig. 3B). Window 17_22 was shown to have a genomewide significant interaction with PRRSV isolate for WG ($P = 1.6 \times 10^{-6}$). This region had the most significant association with WG in the NVSL data ($P = 6.5 \times 10^{-6}$ and 0.8% GV) but was not associated with WG in the KS06 data ($P = 0.01$ and 0.07% GV). Although not genomewide significant, peaks for interactions of SNP genotype with PRRSV isolate were seen at 10_53, 11_43, and 11_52 ($P > 1 \times 10^{-5}$). The 10_52 region, 1 Mb upstream of the isolate interaction region, was associated with WG in the KS06 data ($P = 1.5 \times 10^{-5}$ and 0.4% GV) but was not strongly associated with WG in the NVSL data ($P = 0.001$ and 0.16% GV).

Table 3. Top 1-Mb genomic regions associated with viral load in the KS2006-72109 (KS06) and NVSL 97-7985 (NVSL) trials based on single-SNP and Bayes-B analyses, along with associated candidate genes and previously reported QTL. All analyses included the WUR10000125 region on chromosome 4 in the model

		Single SNP		Bayes-B			
Chromosome	Position, Mb	Maximum $-\log_{10}(P)$	Rank	Genetic variance, %	Rank	Candidate gene	QTL[1]
KS06							
3	57	5.3	1	1.2	1		WBC counts[2]
7	30	5.3	1	0.32	3		Lymphocyte number[3]
7	35	5.0	2	0.82	2		Lymphocyte number[3]
3	53	5.0	2	0.03	25		WBC counts[2]
14	17	5.0	3	0.09	19		C3c concentration[4]
11	28	5.0	3	0.03	25		IFN-γ level, IL-10 level, IL-2 level, TLR 2 level, and TLR 9 level[5]
12	26	4.9	4	0.13	15		IL-10 level, TLR 2 level, and TLR 9 level[5]
15	84	4.7	5	0.06	22		$CD4^+/CD8^+$ leukocyte ratio[3]
9	80	4.7	5	0.02	26	*GNG11*	IgG[2]
14	16	4.3	8	0.31	4		C3c concentration[4]
12	32	3.9	15	0.24	5	*MMD*	IL-10 level, TLR 2 level, and TLR 9 level[5]
7	32	3.5	36	0.24	5		Lymphocyte number[3]
NVSL							
6	79	7.6	1	1.0	3		IL-2 level[5]
6	80	6.8	2	1.0	2		IL-2 level[5]
6	67	6.8	2	0.3	34		IL-2 level[5]
1	247	6.6	3	0.2	35	*DOCK8*	WBC counts[6]
3	68	5.4	4	0.3	34		C3c concentration,[4] IFN-γ level,[5] and IL-10 level[5]
3	128	4.7	5	0.16	21		
1	292	1.9	428	1.76	1		C3c concentration[4] and PRRSV susceptibility[7]
3	72	3.1	32	0.65	4	*STAMBP*	C3c concentration,[4] IFN-γ level,[5] and IL-10 level[5]
15	7	2.3	200	0.6	5		

[1]WBC = white blood cell count; C3c = complement component 3; IFN = interferon; TLR = Toll-like receptor; PRRSV = porcine reproductive and respiratory syndrome virus.

[2]Okamura et al. (2012).

[3]Reiner et al. (2008).

[4]Wimmers et al. (2009).

[5]Uddin et al. (2011).

[6]Wattrang et al. (2005); Cho et al. (2011).

[7]Boddicker et al. (2012).

Neither region on SSC11 with strong isolate interaction effects was strongly associated with WG in either isolate data set ($P > 0.01$ and 0.03 to 0.16% GV).

WUR10000125 Interaction Genomewide Association Study

Information on regions with the strongest interaction effects with genotype at the WUR SNP is presented in Table 6. Figure 4A shows the Manhattan plot of the interaction of WUR with each SNP genotype for VL in the combined data set. A total of 22 SNP in 13 different 1-Mb windows reached genomewide significance for their interaction with genotype at the WUR SNP for VL. These 13 regions were on 5 chromosomes, SSC1_156, and SSC1_171; SSC4_ 96, SSC4_97, SSC4_126, SSC4_131, and SSC4_132; SSC9_138; SSC14_125 and SSC14_137; and SSCX_11. Single nucleotide polymorphisms that interacted with WUR genotype for WG were identified on 1_35, 6_28, 13_199, 18_42, and X_98 ($P < 8 \times 10^{-5}$; Fig. 4B). None of these reached genomewide significance after Bonferroni correction.

Gene Ontology Term Enrichment

PANTHER (Mi et al., 2012) was used to analyze the enrichment of GO terms in lists of genes within 500, 250, or 100 kb of SNP associated with VL or WG with *P*-values based on single-SNP analyses of <0.0001, <0.003, or <0.01. Details for gene lists for each scenario are presented in Table 7. The 10 most enriched GO terms from the PANTHER BPS category were compared between scenarios to deter-

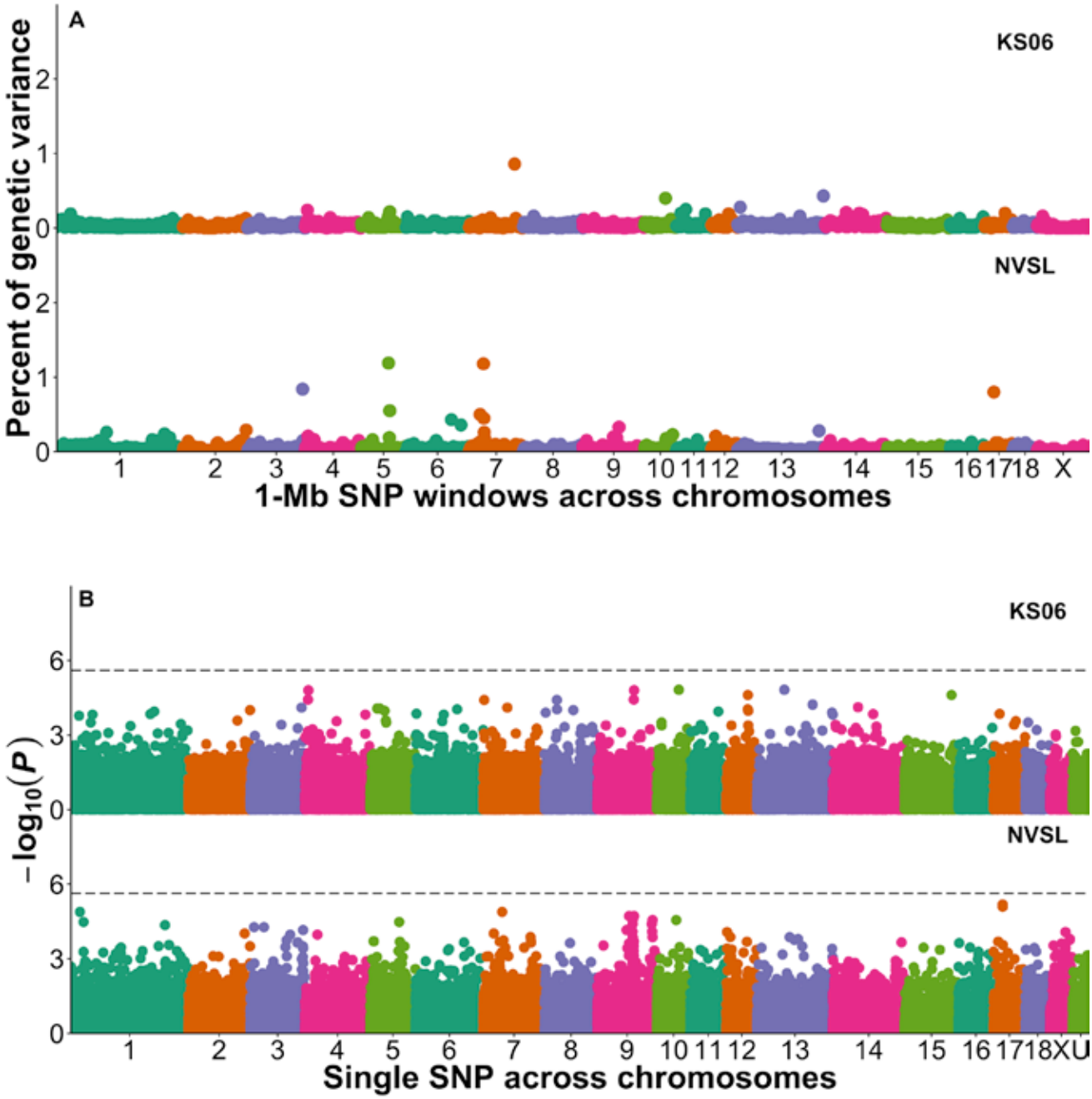

Figure 2. Genomewide association results for weight gain using single-SNP and Bayes-B analyses. (A) Bayes-B results showing the percent of genetic variance explained by 1-Mb nonoverlapping windows of SNP across chromosomes for KS2006-72109 (KS06; top) and NVSL 97-7985 (NVSL; bottom). (B) Single-SNP analysis results showing the $-\log_{10}(P)$ of ordered SNP across chromosomes and for unmapped (U) SNP for KS06 (top) and NVSL (bottom). Dashed lines indicate genomewide significance at a modified Bonferroni corrected *P*-value of 0.05. Results exclude the WUR10000125 region at the end of chromosome 4.

mine the significance threshold and window size for further analyses (Supplemental Tables S2 to S5; see the online version of the article at http://journalofanimalscience.org). Based on consistent enrichment of biologically relevant BPS terms (Supplemental Tables S2 to S5; see the online version of the article at http://journalofanimalscience.org) for genes within 250 kb of SNP with $P < 0.003$, all subsequent analyses were performed using SNP regions that met these criteria.

The most enriched GO slim terms for genes near SNP associated with VL for each PRRSV isolate are presented in Table 8; the complete GO analysis results are presented in Supplemental Table S6 (see the online version of the article at http://journalofanimalscience.org). The 3 BPS and 1 MFS GO terms that were most enriched in the gene list for VL in the KS06 data were also shown to be enriched in the NVSL VL gene list. Consistently enriched BPS GO terms were natural killer cell activation, immune response, and B

Table 4. Top 1-Mb genomic regions associated with weight gain in the KS2006-72109 (KS06) and NVSL 97-7985 (NVSL) trials based on single-SNP and Bayes-B analyses, along with associated candidate genes and previously reported QTL. All analyses included the WUR10000125 region on chromosome 4 in the model

		Single-SNP GWAS[1]		Bayes-B GWAS			
Chromosome	Position, Mb	Maximum $-\log_{10}(P)$	Rank	Genetic variance, %	Rank	Candidate gene	QTL[2]
KS06							
13	59	4.82	1	0.06	22		ADG[3,4]
10	52	4.82	1	0.4	3		ADG,[3,5] BW,[6,7,8] and FCR[9]
4	6	4.80	2	0.09	19		ADG[4,6,10] and BW[6,7,9,11]
9	90	4.80	2	0.03	25		ADG[12] and BW[8]
15	140	4.61	3	0.04	24		ADG[6,13,14]
12	46	4.61	3	0.11	17		BW[6,15] and ADG[4]
4	5	4.43	4	0.24	6		ADG,[4,6,10] BW,[6,7,9] and FCR[9]
9	88	4.43	4	0.03	25		ADG[12] and BW[8]
7	2	4.41	5	0.04	24		ADG[10]
8	26	4.41	5	0.16	12		ADG[4,16,17] and BW[8,15,16]
7	113	1.77	572	0.86	1		ADG[10] and BW[19]
13	214	3.9	14	0.43	2	*MX1*	BW[21]
13	6	2.43	150	0.28	4		ADG[3,5]
11	23	3.41	28	0.25	5		
NVSL							
17	22	5.19	1	0.8	4	*MKKS*	BW[19,22]
7	39	4.89	2	0.17	20	*GLO1*	ADG[6,10,13,17,18,20] and BW[6,8,18,19,20,21]
1	15	4.89	2	0.04	33	*VIP*	BW[16,22] and ADG[4,5]
9	90	4.72	3	0.33	10		BW[8] and ADG[12]
9	73	4.72	3	0.04	33		
9	85	4.56	4	0.13	18		
9	84	4.56	4	0.19	24		
9	145	4.56	4	0.04	33		ADG[24] and BW[15,24]
10	46	4.56	4	0.03	34	*ZEB1*	ADG,[3,4,5] BW,[6,7,8] and FCR[9]
5	71	4.48	5	0.55	5	*ADIPOR2*	BW[15] and ADG[4]
1	22	4.48	5	0.05	32		ADG[4,5,13] and BW[15,19]
5	68	2.28	273	1.19	1		ADG[4] and BW[15,23]
7	35	3.69	21	1.18	2		ADG[6,10,13,17,20] and BW[6,8,18,19,20]
3	138	4.16	9	0.84	3		BW[19] and PRRSV susceptibility[25]

[1]GWAS = genomewide association study.
[2]FCR = feed conversion ratio; PRRSV = porcine reproductive and respiratory syndrome virus.
[3]Knott et al. (1998).
[4]de Keoning et al. (2001).
[5]Liu et al. (2007).
[6]Liu et al. (2008).
[7]Edwards et al. (2008).
[8]Ai et al. (2012).
[9]Jiao et al. (2014).
[10]Paszek et al. (1999).
[11]Choi et al. (2011).
[12]Duthie et al. (2008).
[13]Ruckert and Bennewitz (2010).
[14]Soma et al. (2011).
[15]Yoo et al. (2014).
[16]Beeckmann et al. (2003).
[17]Evans et al. (2003).
[18]Bidanel et al. (2001).
[19]Guo et al. (2008).
[20]Sanchez et al. (2006).
[21]Yue et al. (2003).
[22]Pierzchala et al. (2003).
[23]Schneider et al. (2012).
[24]Cepica et al. (2003).
[25]Boddicker et al. (2014b).

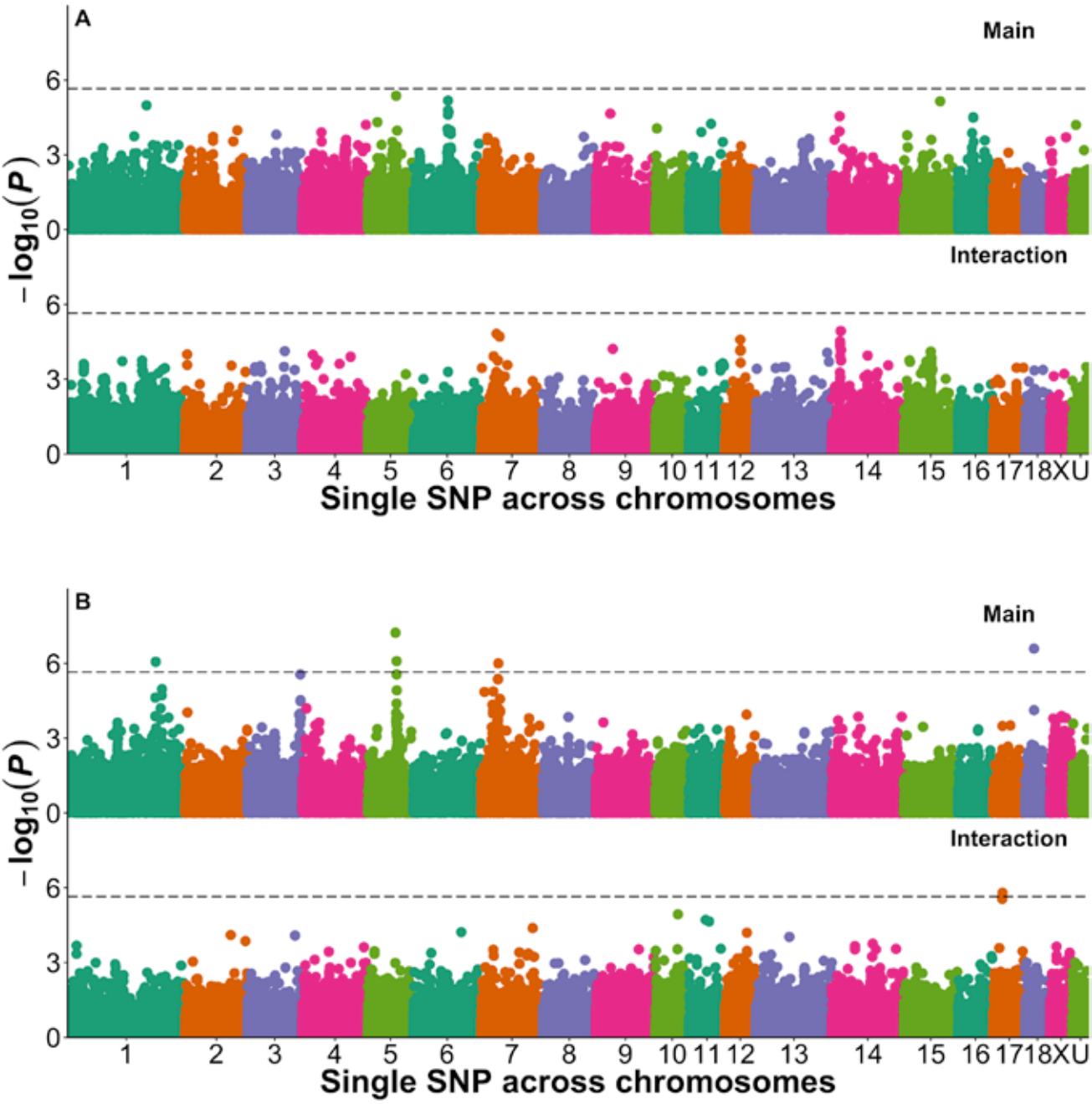

Figure 3. Manhattan plots of $-\log_{10}(P)$ of the main (top) and interaction (bottom) of SNP genotype with porcine reproductive and respiratory syndrome virus isolate for viral load (A) and weight gain (B) across chromosomes and unmapped (U) SNP. Dashed lines indicate genomewide significance at a modified Bonferroni corrected *P*-value of 0.05. Results exclude the WUR10000125 region at the end of chromosome 4.

cell mediated immunity; the MFS GO term that was enriched in both PRRSV isolate gene lists was binding. An additional 7 BPS and 4 MFS GO terms were enriched in the NVSL VL gene list.

The most enriched GO terms for genes near SNP associated with WG are presented in Table 9, with the enriched complete GO terms presented in Supplemental Table S7 (see the online version of the article at http://journalofanimalscience.org). After Bonferroni correction, none of the GO terms were significantly enriched in the KS06 WG gene list. Several metabolic process terms were enriched in the NVSL WG gene list, although only nucleobase-containing compound metabolic process was significantly enriched after Bonferroni correction ($P = 1 \times 10^{-4}$). Genes involved in the molecular function of nucleic acid binding were also enriched in the NVSL WG gene list ($P = 1 \times 10^{-4}$), as was the cholecystokinin receptor (**CCKR**) signaling map pathway ($P = 9.5 \times 10^{-4}$). Table 10 provides information on candidate genes that were identified through GO term enrichment analyses in this study that were also shown to be associated with response to PRRSV infection or with general immune response in

Table 5. Genomewide association study results for the main and interaction effect of SNP genotype and porcine reproductive and respiratory syndrome virus isolate or genotype at the WUR10000125 SNP for viral load and weight gain

Effect	Chromosome	Position, Mb	Largest $-\log_{10}(P)$	Candidate gene
Viral load				
Main	5	70	5.37	
	6	79	5.18	
	15	115	5.15	
	1	205	4.98	
	6	80	4.71	
	9	29	4.65	
Interaction	14	16	4.93	
	7	33	4.82	
	7	39	4.70	
	12	32	4.58	
	9	36	4.21	
Weight gain				
Main	5	68	7.23	
	18	21	6.59	
	5	71	6.09	
	1	238	6.06	
	7	36	6.00	
Interaction	17	22	5.80	*MKKS*
	10	53	4.93	
	11	43	4.71	
	11	52	4.65	
	7	113	4.38	

Table 6. Genomewide association study results for the interaction of SNP genotype and genotype at the WUR10000125 SNP for viral load and weight gain

Chromosome	Position, Mb	Largest $-\log_{10}(P)$	Candidate gene
4	126	8.29	
4	131	7.87	
14	125	6.93	
1	78	6.57	
14	137	6.06	
X	11	6.05	
4	132	5.95	
4	96	5.93	*DEDD*, *SLAMF6*, *CD48*, *CD244*, and *LY9*
1	171	5.81	
4	97	5.78	
4	1	5.76	*LY6H*
9	138	5.71	
1	156	5.69	
13	199	4.88	
X	98	4.48	
18	42	4.25	
6	28	4.25	
1	35	4.09	
13	19	3.98	

previous studies (Badaoui et al., 2013; Dawson et al., 2013; Schroyen et al., 2015).

Genes near SNP whose association with VL had an interaction with PRRSV isolate were enriched for several metabolic process BPS GO terms, whereas genes near SNP whose association with WG had an interaction with PRRSV isolate were enriched for several immune function related GO terms (Table 11). None of the GO terms were consistently enriched in gene lists of SNP that had interaction effects with PRRSV isolate for VL or WG.

Over 1,700 SNP had an interaction effect on VL with genotype at the WUR SNP at $-\log_{10}(P) > 2.5$. There were 4,599 genes within 250 kb of these SNP, which was approximately 4 times as many genes as in the lists created with these criteria for VL and WG in each PRRSV isolate data set (Table 7). We would not expect so many regions to have differential effects on VL in animals with different genotypes at the WUR SNP, and therefore, this large gene list likely contains many false positives. Furthermore, an extremely large gene list decreases power to detect GO term enrichment. Therefore, we increased the significance threshold of SNP association for the interaction with WUR for VL and created a list of genes within 250 kb of the 383 SNP with $-\log_{10}(P) > 4$, resulting in a similar number of genes (1,048) as the other lists analyzed in this study. This list was enriched for enzymatic GO terms (Table 12). For WG, genes near 180 SNP with interactions with WUR at $-\log_{10}(P) > 2.5$ were not enriched for any BPS or molecular function GO terms.

DISCUSSION

We used Bayesian and single-SNP GWAS methods to identify genomic regions associated with host response to experimental infection with 1 of 2 different isolates of PRRSV. Several genomic regions were found to be consistently associated with host response traits across methods, but single-SNP analysis was better able to detect small effects than Bayes-B. Bayes-B analysis simultaneously fits all SNP as random effects, whereas single-SNP analysis fits each SNP one at a time as a fixed effect. The single-SNP method that was used in this study also allowed for other random effects, whereas the current version of GenSel, which was used for the Bayes-B analyses, does not allow random effects other than SNP effects. Neither Bayes-B nor single-SNP analysis found regions that were associated with either trait for both PRRSV isolates, which was unexpected given the high genetic correlations

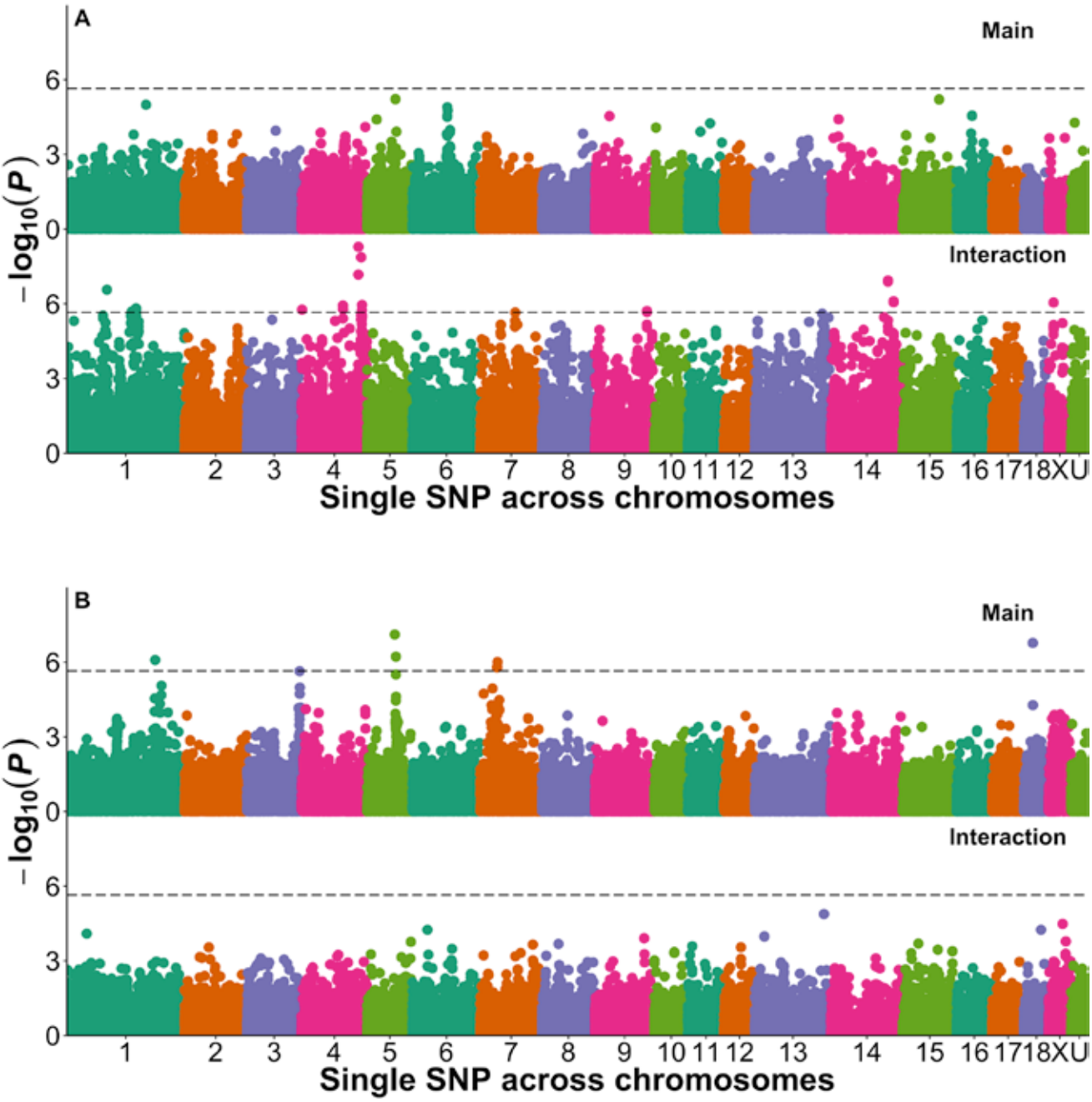

Figure 4. Manhattan plots of $-\log_{10}(P)$ of the main (top) and interaction (bottom) of SNP genotype with genotype at the WUR10000125 SNP for viral load (A) and weight gain (B) across chromosomes and unmapped (U) SNP. Dashed lines indicate genomewide significance at a modified Bonferroni corrected *P*-value of 0.05.

between isolates estimated by Hess et al. (2016; 0.86 ± 0.19 for VL and 0.86 ± 0.27 for WG). The lack of common associated regions may be due to the genetic correlation resulting from many very small pleiotropic effects that were not detected by GWAS.

Genotype at the WUR SNP was fitted as a fixed effect in all analyses to account for the highly significant effect of the SSC4 QTL. Very few SNP in other genomic regions reached genomewide significance in the single-SNP analysis. Also, the GV explained by 1-Mb windows in the Bayesian analyses was very small, and their associations could be considered spurious if not for our results from functional analysis of neighboring genes, which showed enrichment of highly relevant immune response pathways. Traits related to immune response and WG are very complex and likely involve actions and interactions of a large number of genetic loci. Creating gene lists from genes nearby SNP associated with each trait and performing GO term enrichment of these lists provided confirmation of the biological significance of this collective

Table 7. Numbers of genes located within 100, 250, or 100 kb of SNP associated with viral load or weight gain at 3 $-\log_{10}(P)$ thresholds (2, 2.5, and 3) for the KS2006-72109 (KS06) and NVSL 97-7985 (NVSL) isolates

PRRSV[1] isolate	$-\log_{10}(P)$ threshold	No. of SNP with $-\log_{10}(P)$ > threshold	No. of nonoverlapping regions for each interval size			No. of pig Ensembl ID[2] (No. of Ensembl ID with GO annotation)		
			500	250	100	500	250	100
Viral load								
KS06	3	215	157	166	187	1,576 (1,284)	844 (706)	401 (332)
	2.5	456	305	347	393	3,304 (2,723)	937 (781)	443 (369)
	2	1,053	567	686	849	6,445 (5,305)	3,828 (3,170)	1,184 (1,557)
NVSL	3	115	88	95	102	1,014 (835)	556 (461)	263 (218)
	2.5	303	208	239	267	2,418 (1,991)	1,390 (1,140)	660 (553)
	2	810	467	566	667	5,347 (4,441)	3,166 (2,645)	1,542 (1,323)
Weight gain								
KS06	3	116	93	98	106	931 (762)	514 (423)	254 (210)
	2.5	245	193	208	223	2,048 (1,686)	1,108 (916)	510 (430)
	2	720	476	554	625	5,269 (4,306)	3,024 (2,466)	1,451 (1,194)
NVSL	3	169	118	127	137	1,173 (942)	592 (486)	287 (238)
	2.5	378	259	282	311	2,812 (2,291)	1,510 (1,254)	722 (613)
	2	958	516	639	770	6,075 (4,908)	3,555 (2,895)	1,745 (1,439)

[1]PRRSV = porcine reproductive and respiratory syndrome virus.

[2]ID = identification; GO = gene ontology. Ensembl.

set of associations, including associations that did not reach restrictive significance thresholds.

Viral Load

Genomewide association study revealed regions on 3_57, 7_30, 7_35, 9_80, 11_28, 12_26, 14_17, and 15_84 to be associated with VL in the KS06 data and on 1_247, 1_292, 3_68, 3_72, 3_128, 6_67, 6_69, 6_80, and 15_7 to be associated with VL in the NVSL data (Table 3). Immune-related QTL were found in 19 of the top 21 regions identified for VL in either PRRSV isolate; selected regions are further described in the following.

Table 8. Most enriched gene ontology (GO) terms for the gene lists for viral load for the KS2006-72109 (KS06) and NVSL 97-7985 (NVSL) isolates

Category	GO term	No. of genes in list	Fold enrichment	*P*-value	Bonferroni *P*-value
KS06					
Biological process slim	Natural killer cell activation	19	4.9	2.79×10^{-8}	6.28×10^{-6}
	Immune response	37	1.97	1.11×10^{-4}	0.025
	B cell mediated immunity	15	2.64	7.91×10^{-4}	0.18
Molecular function slim	Binding	244	1.25	5.22×10^{-5}	9.08×10^{-3}
NVSL					
Biological process slim	Regulation of vasoconstriction	13	4.92	4.1×10^{-6}	9.23×10^{-4}
	Immune response	51	1.86	2.99×10^{-5}	6.73×10^{-3}
	Metabolic process	484	1.15	6.2×10^{-5}	0.014
	Lysosomal transport	11	4.27	7.85×10^{-5}	0.0177
	Primary metabolic process	407	1.17	9.57×10^{-5}	0.0215
	Endocytosis	35	1.93	2.44×10^{-4}	0.0549
	Receptor-mediated endocytosis	23	2.27	3.26×10^{-4}	0.0734
	Biological regulation	246	1.23	3.43×10^{-4}	0.0772
	B cell mediated immunity	20	2.41	3.78×10^{-4}	0.0851
	Natural killer cell activation	15	2.65	7.68×10^{-4}	0.173
Molecular function slim	Serine-type endopeptidase inhibitor activity	20	4.89	1.30×10^{-8}	2.26×10^{-6}
	Binding	356	1.25	1.36×10^{-6}	2.37×10^{-4}
	Peptidase inhibitor activity	28	2.73	3.11×10^{-6}	5.41×10^{-4}
	Enzyme inhibitor activity	35	2.24	1.37×10^{-5}	2.38×10^{-3}
	Receptor binding	74	1.53	2.79×10^{-4}	4.85×10^{-2}

Table 9. Most enriched gene ontology (GO) terms for the gene lists for weight gain for the KS2006-72109 (KS06) and NVSL 97-7985 (NVSL) isolates

Category	GO term	Fold enrichment	No. of genes in list	*P*-value	Bonferroni *P*-value
KS06					
Biological process slim	Antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	4.95	7	6.25×10^{-4}	0.147
NVSL					
Biological process slim	Nucleobase-containing compound metabolic process	1.25	238	1.38×10^{-4}	0.031
	Metabolic process	1.11	514	1.5×10^{-3}	0.34
Molecular function slim	Nucleic acid binding	1.28	200	1.6×10^{-4}	0.028
	DNA binding	1.35	130	3.52×10^{-4}	0.061
	Binding	1.16	365	5.67×10^{-4}	0.099
PANTHER[1] pathway	CCKR signaling map	2.29	19	9.52×10^{-4}	0.15

[1]Mi et al. (2012).

Apart from the WUR region, the region on 1_292 was the only genomic region previously identified by Boddicker et al. (2014b) as being associated with VL in the first 8 trials of NVSL-infected piglets, using the Bayes-B method, which is the same GWAS method that identified this QTL in this study; single-SNP GWAS did not show a strong association for this region. The 6_79 and 6_80 region was shown to be associated with NVSL VL by both GWAS methods. Uddin et al. (2011) identified a QTL in this same region for IL-2 levels in serum of pigs after vaccination. Interleukin 2 has been shown to play an important role in T cell activation, proliferation, and differentiation (Bachmann and Oxenius, 2007). Genes in the 1_247 region, which was significantly associated with VL in both isolate data sets, include *DOCK8* (*dedicator of cytokinesis 8*) which, when mutated in humans, causes combined immunodeficiency (Engelhardt et al., 2009). *DOCK8* deficiency in a mouse knockout model leads to a loss of circulating natural killer (**NK**) T cells (Crawford et al., 2013). *DOCK8* was also a member of a gene module whose expression was correlated with PRRSV viremia at 4 and 7 dpi (Schroyen et al., 2015). A QTL for white blood cell counts has also been

Table 10. Candidate genes identified by gene ontology term enrichment for viral load and weight gain for the KS2006-72109 (KS06) and NVSL 97-7985 (NVSL) isolates that were shown to be associated with response to porcine reproductive and respiratory syndrome virus infection or general immune response in previous studies

Candidate gene	Evidence[1]	Reference
KS06 viral load		
MYO5A, *CFI*, *FCRL1*, *FCRL4*, and *SLA-DQA1*	Members of gene modules correlated with VL	Schroyen et al. (2015)
TXNDC9 and *IKBKB*	Differentially expressed in blood	Schroyen et al. (2015)
SLAMF9	PRRS-specific response	Badaoui et al. (2013)
GBP1	General immune response	Badaoui et al. (2013)
NVSL viral load		
CCRL1, *CLEC1A*, *HP*, *SEMA6C*, *SEZ6L*, *TMEM176A*, *TMEM176B*, *CCRL1*, and *CLEC1A*	Members of gene modules correlated with VL	Schroyen et al. (2015)
RHOC		Dawson et al. (2013)
HSPB1	General immune response	Badaoui et al. (2013)
KS06 weight gain		
CDIPT, *SLAMF9*, and *NMT1*	PRRS-specific response	Badaoui et al. (2013)
NMT1 and *TAP1*		Dawson et al. (2013)
SBF1 and *TAP1*	Differentially expressed	Schroyen et al. (2015)
NVSL weight gain		
SSU72 and *TOP2A*	General immune response	Badaoui et al. (2013)
TOP2A, *CDA*, *CDK6*, *EPS15*, *FKBP3*, *IER3*, *INPP5F*, *NFIL3*, *PSMD12*, *RGS10*, *RHOQ*, *TAB2*, *TCEB3*, and *WBSCR22*	PRRS-specific response	Schroyen et al. (2015)
ALPK2, *CEBPB*, *CPSF3*, *NEK9*, *TFAP2A*, *TXNDC9*, and *DDX6*	Differentially expressed	Schroyen et al. (2015)
EIF5B, *UBE2V1*, *CITED2*, *LAPTM4A*, *LCN2*, and *PLA2G4A*	Members of modules correlated with weight gain	Schroyen et al. (2015)
AIFM1, *ATP11C*, *CNOT7*, *FUNDC1*, *HIST1H2BH*, *MOBKL3*, *MTRF1L*, and *PLA2G6*		Dawson et al. (2013)

[1]VL = viral load; PRRS = porcine reproductive and respiratory syndrome.

Table 11. Most enriched gene ontology (GO) terms for the interaction with porcine reproductive and respiratory syndrome virus isolate for viral load and weight gain

Category	GO term	Fold enrichment	No. of genes in list	*P*-value	Bonferroni *P*-value
Viral load					
Biological process slim	Nucleobase-containing compound metabolic process	1.32	260	1.47×10^{-6}	3.31×10^{-4}
	Primary metabolic process	1.18	468	1.15×10^{-5}	2.59×10^{-3}
	Metabolic process	1.15	552	1.82×10^{-5}	4.10×10^{-3}
	Transcription from RNA polymerase II promoter	1.42	129	5.31×10^{-5}	1.19×10^{-2}
	Transcription, DNA-dependent	1.36	139	1.83×10^{-4}	4.12×10^{-2}
	Regulation of transcription from RNA polymerase II promoter	1.43	98	3.13×10^{-4}	7.04×10^{-2}
Molecular function slim	Nucleic acid binding	1.31	212	2.7×10^{-5}	4.63×10^{-3}
	Nucleotide kinase activity	3.94	11	1.57×10^{-4}	0.027
	Sequence-specific DNA binding transcription factor activity	1.41	107	2.81×10^{-4}	0.05
Weight gain					
Biological process slim	Antigen processing and presentation	>5	11	2.36×10^{-6}	5.31×10^{-4}
	Pattern specification process	3.02	19	2.94×10^{-5}	6.62×10^{-3}
	Response to interferon-gamma	>5	8	7.24×10^{-5}	0.016
	Antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	>5	7	1.41×10^{-4}	0.032
	Skeletal system development	2.63	18	2.52×10^{-4}	0.057
	DNA binding	1.35	130	3.52×10^{-4}	0.061
	Binding	1.16	365	5.67×10^{-4}	0.099

identified in the 1_247 region (Wattrang et al., 2005; Cho et al., 2011). *GNG11*, located at 9_80, which was significantly associated with VL in the KS06 data set and WG in both data sets, plays a role in cellular senescence and shows expression changes during cellular stress (Hossain et al., 2006). *GNG11* has also been shown to have differential expression in response to PRRSV infection (Badaoui et al., 2013). The 9_80 region also contained a QTL for IgG level (Okamura et al., 2012). *STAMBP* (*AMSH*), located at 3_72, which was significant for VL in the KS06 data set, interacts with STAM and other proteins involved in cytokine signaling (Tanaka et al., 1999). Quantitative trait loci for complement component 3 concentration (Wimmers et al., 2009) and interferon (**IFN**)-γ and IL-10 levels (Uddin et al., 2011) have been found in the 3_72 region. The 11_28 region identified for KS06 VL using single-SNP analyses contains a QTL for IFN-γ, IL-10, and Toll-like receptor 9 (**TLR9**) levels in blood after vaccination (Uddin et al., 2010, 2011).

Genomic regions associated with VL did not overlap between PRRSV isolates, but genes near associated SNP were enriched for the same GO terms. This indicates that associated SNP are in linkage disequilibrium with variants that are involved in similar biological processes or functions across the PRRSV isolates. For both PRRSV isolates, genes near SNP associated with VL were enriched for natural killer cell activation, immune response, and B cell mediated immunity BPS GO terms. The activity of NK cells, specifically via

Table 12. Most enriched gene ontology (GO) terms for the interaction with genotype at the WUR10000125 SNP for viral load

Category	GO term	Fold enrichment	No. of genes in list	*P*-value	Bonferroni *P*-value
Viral load					
Biological process slim	Blood coagulation	3.42	18	9.75×10^{-6}	2.19×10^{-3}
	Proteolysis	1.73	45	3.45×10^{-4}	0.078
Molecular function slim	Serine-type endopeptidase inhibitor activity	>5	18	3.17×10^{-9}	5.52×10^{-7}
	Peptidase inhibitor activity	3.45	26	9.34×10^{-8}	1.63×10^{-5}
	Amylase activity	>5	6	2.53×10^{-6}	4.4×10^{-4}
	Hydrolase activity, hydrolyzing *O*-glycosyl compounds	>5	9	1.46×10^{-5}	2.54×10^{-3}
	Enzyme inhibitor activity	2.45	28	2.15×10^{-5}	3.74×10^{-3}
	Peptidase activity	1.9	41	9.9×10^{-5}	0.017

production of IFN-γ, is thought to play an important role in reduction and clearance of PRRSV (Wesley et al., 2006). Nineteen and 15 genes in the gene lists for VL in KS06 and NVSL, respectively, were involved in NK cell activation. A region on SSC5_64 and SSC5_65 contained several genes in one or both VL lists, including *LY49* (*KLRA1*), *KLRD1*, *KLRF1*, *KLRG1*, *CLECL1*, *CLEC1A*, *CLEC1B*, *CLEC7A*, and *CLEC12A*. *LY49* encodes a receptor found on NK cells that is involved in response to viral infection (Brown et al., 2001), possibly via their role in apoptosis (Berry et al., 2013). *KLRD1*, *KLRF1*, and *KLRG1* are molecules expressed on NK cells (Li and Mariuzza, 2014) that have the ability to recognize non-self cells that do not express major histocompatibility complex (**MHC**) Class I (Li et al., 2009). Members of the it *CLEC* gene family are C-type lectin receptors that are found on antigen presenting cells and act to recognize pathogens and promote cell–cell interactions for effective immune responses (McGreal et al., 2005; Kanazawa, 2007).

Viral load gene lists also included *SLAMF9*, *JAK3*, *FCAR*, *FCRL3*, *IKBK*, *PRKRA* (*PACT*), *PECAM1*, and the guanylate-binding protein (**GBP**) family genes, all annotated with the immune response GO term. The 7 genes in the porcine GBP family are in the SSC4 QTL region, which was previously shown to have an effect on VL (Boddicker et al., 2012). Recently, a putative causative mutation for the SSC4 QTL was identified in the *GBP5* gene (Koltes et al., 2015). IKBK is a kinase that is part of the pathway responsible for initiation of the NF-κB signaling cascade, which serves as one of the host's first responses after viral invasion (Amaya et al., 2014). *IKBK* has also been shown to be differentially expressed in response to infection with the NVSL PRRSV isolate (Schroyen et al., 2015). *PACT* recognizes RNA viruses and acts with *RIG-I* to stimulate type 1 IFN production during an immune response against a virus (Kok et al., 2011; Kok and Jin, 2013). *SLAMF9* is a member of the signaling lymphocyte activation molecule receptor family, which contains NK cell receptors that affect T cell responses to viral infection (Waggoner and Kumar, 2012). *SLAMF9* has been shown to be differentially expressed in response to PRRSV infection in an independent data set (Badaoui et al., 2013). *PECAM1* mediates adhesion of leukocytes to other cells and permits movement of leukocytes through endothelial walls (Proust et al., 2014).

Weight Gain

Genomewide association study identified regions on 7_113, 10_52, 13_59, and 13_214 to be associated with WG in the KS06 data and on 1_15, 5_68, 5_71, 7_35, 7_39, 10_46, and 17_22 to be associated with WG in the NVSL data. Quantitative trait loci for traits related to production, such as ADG and WG, were found in 24 of the top 25 regions associated with WG in either PRRSV isolate.

The *MX1* gene is located in the 13_214 region and has been shown to be associated with the initial defense mechanisms used by macrophages on PRRSV infection (Chung et al., 2004). Furthermore, variations in the *MX1* promoter have been shown to be associated with increased *MX1* gene expression, which could be expected to result in heightened immune response to PRRSV and possibly more resistant pigs (Li et al., 2015). The 1_15 region contains the *VIP* (*vasoactive intestinal peptide*) gene; the VIP pathway has been shown to be associated with obesity in humans (Liu et al., 2010). *GLO1*, located in 7_39, has been shown to be associated with BW in mice (Wuschke et al., 2007). The 17_22 region contains an interesting candidate gene, *MKKS*, as *MKKS*-null mice were shown to weigh more and consume a greater amount of food compared with wild-type littermates (Fath et al., 2005). In addition, certain *MKKS* haplotypes have been shown to be associated with obesity and metabolic syndrome in a Greek human population (Rouskas et al., 2008). Quantitative trait loci for pig weaning weight (Guo et al., 2008) and live weight at slaughter (Pierzchala et al., 2003) have also been identified in this region on SSC17. Nagamine et al. (2003) mapped several growth related QTL to SSC7.

Similar to results for VL associations, none of the identified genomic regions were associated with WG for both PRRSV isolates. We also did not see overlap of overrepresented GO terms across PRRSV isolates for WG. The WG phenotype analyzed in this study is more accurately described as WG after infection with the PRRSV; therefore, we could expect the severity of PRRSV infection to have a negative relationship with WG. Genes near SNP associated with WG in the NVSL data were enriched for several metabolic process BPS, several binding related MFS GO terms, and the CCKR signaling map pathway. Genes in the metabolic process GO term included *FOXO3*, *INPP5F*, *PRKCQ*, *LIPC*, *BMP7*, *HMGA1*, and *NLK*. The gene *FOXO3* has been shown to be involved in growth retardation of piglets born to sows fed low-protein diets during gestation and lactation (Jia et al., 2015). Different *INPP5F* genotypes have been identified to be associated with ADG in purebred Yorkshire gilts (Zhou et al., 2009). In addition, a meta-analysis by Badaoui et al. (2013) showed *INPP5F* to have differential expression during PRRSV infection. *PRKCQ* has been shown to be associated with obesity in mice, through insulin resistance (Serra et al., 2003; Gao et

al., 2007). LIPC is an enzyme that is important in energy homeostasis and has been shown to affect WG in mice (Chiu et al., 2010). *BMP7* affects brown adipose tissue development and plays a role in obesity (Tseng et al., 2008; Townsend et al., 2012). *HMGA1* has been shown to be associated with measures of fat deposition in pigs (Kim et al., 2004) as well as with body mass index in Hispanic women (Graff et al., 2013). *NLK* variants have been shown to be associated with fat body mass (Pei et al., 2013). The CCKR (cholecystokinin receptor) pathway is activated in response to food consumption and acts to regulate gastrointestinal secretions and motility (Wank, 1995). Polymorphisms in the *cholecystokinin type A receptor* gene have been shown to be associated with feed consumption and growth rate traits in pigs (Houston et al., 2006).

Boddicker et al. (2012, 2014a,b) estimated moderately negative phenotypic and genetic correlations between WG and VL in the NVSL data; phenotypic correlation estimates ranged from −0.25 to −0.29 and genotypic correlation estimates ranged from −0.31 to −0.46. We did not identify any associated genomic regions that overlapped between WG and VL for either PRRSV isolate; however, we did find that the NVSL gene lists for WG and VL were both enriched for the binding molecular function GO term. In addition, the KS06 WG gene list was enriched for antigen processing and presentation of peptide or polysaccharide via MHC class II.

Joint Genomewide Association Study

Genomic regions with the most significant main effects for VL across the 2 PRRSV isolates were at 5_70, 6_79, 6_80, 15_115, 1_205, and 9_29. Candidate genes in these regions included *ERC1* and *ATG1*. *ERC1* has been shown to play a role in the in vitro production of herpes simplex virus 1 in HeLa cells (Johns et al., 2014). The autophagy-related gene, *ATG1*, has been shown to be involved in lung inflammation during influenza virus infection (Lu et al., 2016). For WG, the genomic regions with the strongest main effects were at 5_68, 18_21, 5_71, 1_238, and 7_36. Regions associated with VL on 14_16, 7_33, 7_39, 12_32, and 9_36 had the strongest interaction effect with PRRSV isolate; for association with WG, SNP at 17_22, 10_52, 11_43, 11_52, and 7_113 showed interaction effects with PRRSV isolate. Several of these regions were associated with these respective traits in GWAS results using data from only 1 PRRSV isolate.

WUR10000125 Interaction Genomewide Association Study

The most notable result from the interaction GWAS was the identification of 13 regions with genomewide significant associations for the interaction with genotype at the WUR SNP for VL. Candidate genes in these regions include *DEDD*, *SLAMF6* (*NTBA*), *CD48*, *CD244* (*2B4*), and *LY9* at SSC4_96 and *LY6H* at SSC4_1. The *DEDD* (*death effector domain containing*) gene is involved in apoptosis (Alcivar et al., 2003). *SLAMF6* plays a role in the activation of NK and T cells (Flaig et al., 2004; Valdez et al., 2004). *CD244* is a receptor of NK cells whose ligand is *CD48*, expressed on T cells; both of these molecules are involved in lymphocyte activity (Pacheco et al., 2013; Kis-Toth and Tsokos, 2014). *LY9* is another member of the signaling lymphocytic activation molecule (SLAM) family involved in mediating innate T cell function (Sintes et al., 2013).

We expected to see enrichment of genes in GO terms related to GBP family protein functions in the gene lists created from the interactions of SNP genotypes with genotype at the WUR SNP, which is in very high linkage disequilibrium with a putative causative mutation in the *GBP5* gene (Koltes et al., 2015). Instead, results showed enrichment for blood coagulation, proteolysis, and several peptidase activity GO terms. The IFN-inducible GBP family genes are annotated instead with cytokine signaling, interferon signaling, cellular response to interferon γ, defense response to virus, and metabolic process GO terms. Therefore, it is not clear how genes involved in blood coagulation, proteolysis, and peptidase activity functions could have differential effects based on genotype at the WUR SNP. Furthermore, there were no genes in the VL or WG gene list for interaction with the WUR SNP that were known to have protein interactions with any member of the GBP protein family.

We also assessed enrichment of the WUR interaction gene lists for genes shown to have a different expression pattern over time between WUR genotypes in RNA sequencing data collected on 16 pigs infected with the NVSL isolate (Schroyen et al., 2015). Seventeen and 13 of the 516 genes that differed between WUR genotypes were within 250 kb of SNP whose association with VL and WG, respectively, had an interaction with genotype at the WUR SNP at $-\log_{10}(P) > 4$ (VL) or $-\log_{10}(P) > 2.5$ (WG; data not shown). These 516 genes were not overrepresented in either of our WUR interaction gene lists ($P > 0.05$).

Value of Functional Annotation of Genomewide Association Study Results

Single-SNP analyses revealed 0, 2, 0, and 0 mapped genomic regions that reached genomewide significance for KS06 VL, NVSL VL, KS06 WG, and NVSL WG, respectively. Few candidate genes were identified in regions surrounding the most significantly associated SNP. Therefore, we created gene lists using a relaxed significance threshold for SNP and assessed enrichment of genes assigned to GO annotation terms in these lists. Initially, we analyzed genes within 100, 250, or 500 kb of SNP associated with the trait at thresholds of $P < 0.001$, $P < 0.003$, or $P < 0.01$. We considered the top 10 enriched BPS GO terms for each scenario and chose the middle stringency threshold ($P < 0.003$) and medium-sized window (250 kb) for further analyses based on consistency of enriched terms across combinations of variables and biological relevance of the enriched GO terms.

Several biologically relevant GO terms were shown to be enriched in our gene lists, which provided a larger set of candidate genes, consisting of genes with the given GO term annotation within 250 kb of SNP associated with the trait at $P < 0.003$. Several of the genes identified through this method were differentially expressed in response to PRRSV infection in other studies. The use of annotation information provided greater insight into genomic regions that may play a role in piglet response to PRRSV. This method was especially useful in interpreting the complicated immune response–related traits analyzed here, for which many genes may have small effects on phenotype. Such small effects may not be detected when considered individually, but by grouping the effects of genes that play a role in the same biological function or pathway, larger effects on the phenotype may be detected. These results may be useful for creating subsets of SNP to be used in genomic prediction or selection, in an effort to reduce noise of unrelated or unassociated SNP, while keeping SNP that have small effects on phenotype.

We also tested enrichment of our lists for genes that were shown to be differentially expressed in the blood of pigs selected from 3 NVSL trials at 4 or 7 dpi compared with 0 dpi by Schroyen et al. (2015). The Pigoligoarray (Steibel et al., 2009) was used to measure gene expression in the blood of 100 pigs that were selected based on their assignment to 1 of 4 phenotypic groups after infection with the NVSL isolate (high VL with high WG, high VL with low WG, low VL with high WG, and low VL with low WG). Schroyen et al. (2015) found that 239 and 165 genes were differentially expressed (false discovery rate = 0.10) at 4 and 7 dpi, respectively, compared with 0 dpi (supplemental tables 1 and 2 found in Schroyen et al., 2015). We did not find a statistically significant overrepresentation of these differentially expressed genes in any of our analyzed gene lists, but several of the candidate genes that we identified (Table 9) were present in the differentially expressed list. Furthermore, some genes in our gene lists were members of gene modules whose overall expression was correlated with either VL or WG, as shown in Table 9 (Fig. 3 in Schroyen et al., 2015).

We also compared our gene lists with results from 2 meta-analyses of pig immune response: 1) Dawson et al. (2013), who expanded the pig immunome annotation through analysis of data from 188 Affymetrix 24K microarray chips (Affymetrix, Inc., Santa Clara, CA) from several studies that used in vivo or in vitro immune stimulation, and 2) Badaoui et al. (2013), who used 779 chips from 29 studies to identify genes, pathways, and biological functions involved in immune response of pigs. Our gene lists contained 1 (VL) and 10 (WG) genes that were members of a cluster of 511 gene transcripts that were differentially expressed in response to immune stimuli and that had enriched GO terms related to immune function (Supplemental Table 6 in Dawson et al., 2013). The 139 differentially expressed genes for general immune response identified by Badaoui et al. (2013) included 2 genes that were in our VL list and 2 genes that were in our WG list, whereas their 537 differentially expressed genes for response to PRRSV infection included 1 gene from our VL list and 17 genes from our WG list (Supplemental file 10, Table S8 in Badaoui et al., 2013). Our lists, however, were not enriched for these sets of differentially expressed genes at $P = 0.05$.

Conclusions

We found several genomic regions associated with response to experimental infection with 1 of 2 isolates of the PRRSV that were consistently identified using both single-SNP and Bayesian GWAS methods. Apart from the previously identified QTL on chromosome 4 associated with a mutation in the *GBP5* gene, we did not find genomic regions that were significantly associated with piglet response for both PRRSV isolates, despite high estimates of genetic correlations between host response to these 2 isolates; however, we did find that genes near associated SNP were enriched for the same GO terms for both PRRSV isolates and that these enriched terms were highly relevant to immune response to infection. This indicates that similar biological mechanisms are involved in the response to both PRRSV isolates through the actions of many genes but that apart from the *GBP5* gene, the effects of host response QTL are small and numerous. These

results suggest the need for genomic prediction methods to select for host response to porcine reproductive and respiratory syndrome.

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