Whole-Genome Scan and Validation of Regions Previously Associated with PRRS Antibody Response and Growth Rate using Gilts Under Health Challenge in Commercial Settings

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ABSTRACT: The genomic basis of average daily gain (ADG) and Porcine Respiratory and Reproductive Syndrome (PRRS) IgG-antibody measured by ELISA sample-to-positive ratio (S/P), was assessed in crossbred replacement gilts entering commercial farms. S/P at time of entry (S/P-d0), after acclimation (S/P-Acc), and parity 1 (S/P-Par), and ADG during acclimation were analyzed. All traits had low heritability, except S/P-Acc (0.47). Previously identified candidate SNPs for S/P on SSC7 (MARC, ASGA, M1GA, and ALGA) were validated, but effects of the SSC4 WUR SNP on S/P and growth were not significant. Two regions on SSC7 previously associated with S/P, that harbor the four candidate SNPs, were validated. One of the SSC7 regions lost its association when candidate SNPs were included in the model, indicating that these SNPs captured the effect of the region on the trait. Novel regions were associated with ADG, S/P-d0, and S/P-Acc.

Keywords: Porcine Reproductive and Respiratory Syndrome; GWAS; genetics

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is the most economically significant disease impacting pig production in North America, Europe, and Asia (Rowland et al. (2012)). Recent reports suggest opportunities to exploit host genetics against the PRRS virus (PRRSV). Boddicker et al. (2012) found a QTL on *Sus scrofa* chromosome (SSC) 4 associated with PRRS viremia and weight gain in growing pigs after experimental challenge. Most of the genetic variation for these traits was accounted for by one Single Nucleotide Polymorphism (SNP), WUR10000125. In addition, Serão et al. (2014) reported QTLs on SSC2, SSC7, and SSC14 associated with PRRSV antibody response, measured as sample-to-positive (S/P) ratio, in a reproductive PRRSV outbreak herd. In particular, four SNPs on SSC7, including two in the Major Histocompatibility Complex (MHC), were significantly associated with S/P ratio, explaining approximately 45% of the genetic variance (SNPs MARC0058875, ASGA0032151, M1GA0025482 and ALGA0045692).

Although these findings provide insights into host genetics responses to PRRSV, information on the effect of PRRSV on replacement gilts under commercial conditions is scarce. Therefore, the objective of this study was to perform a genome-wide association study (GWAS) for growth performance and PRRSV IgG response in gilts following standard acclimation procedures, and to validate genomic regions and SNPs previously associated with PRRSV.

Materials and Methods

Selection of herds and acclimation procedures. Commercial herds with a consistent gilt introduction protocol were selected for this study. Consultation with the veterinarian of each commercial herd was undertaken prior to inclusion in the study to ensure that a significant health challenge existed. A total of 18 herds were used in this analysis. Non-pregnant gilts were introduced in the herd in groups (up to 7) of 10 to 47 gilts. The combination between herd and group was used to define contemporary groups (CG; 45 levels).

A passive acclimation protocol was used (i.e. no direct challenge for any disease). Gilts were directly introduced following the normal acclimation procedures used by the cooperating herd. Fifteen of 18 farms vaccinated gilts against PRRSV upon arrival, using a modified live virus vaccine.

Source of the data. Data on 923 F1 gilts from 13 supplier herds were used in this study. Individual weights were taken twice: at day 0, when gilts were introduced in the herd, and after acclimation (40.1±14 days). Blood samples were collected on the same days and also at first parity weaning, for genotyping and PRRSV ELISA (IDEXX PRRS X3, Laboratories Inc.; Westbrook, Maine). The ELISA results are reported as sample-to-positive ratio (S/P). Phenotypes analyzed were: Average daily gain during the acclimation period (ADG), and S/P on day 0 (S/P-d0), after acclimation (S/P-Acc), and at first parity (S/P-Par). Descriptive statistics and proportion of PRRSV antibody positive animals across periods are in Table 1.

Table 1. Mean, standard deviation (SD) and number of individuals (*n*) for body weight (BW) and PRRS ELISA sample-to-positive ratio (S/P), and proportion of PRRS virus positive (PRRSV+) animals across time periods.

	BW, kg			S/P			
Period	mean	SD	n	mean	SD	n	PRRSV+[§]
Day 0	119.8	25.1	886	0.12	0.37	613	0.07
Acclimation	141.5	25.9	886	1.37	0.62	518	1
Parity 1		-		1.01	0.95	480	0.34

[§]PRRSV+, S/P ratio ≥ 0.4.

Genotype data. All 923 gilts were genotyped using the Illumina PorcineSNP60 BeadChip. Of the 61,565 SNPs, 8,710 were removed due to poor quality (GenCall < 0.2, SNP call rate < 95%, and MAF < 1%). The remaining 52,855 SNPs had a total genotyping call rate of 99.92%.

Candidate SNPs. Individual genotypes of five SNPs previously associated (Boddicker et al. (2012); Serão et al. (2014)) with host response to PRRS virus infection were obtained from the genotype data to be included in the statistical models. These SNPs were: WUR10000125 (WUR) on SS4, and MARC0058875 (MARC), ASGA0032151 (ASGA), M1GA0025482 (M1GA), and ALGA0045692 (ALGA) on SSC7.

Heritability and genome-wide associations. Bayesian genomic prediction methods were used to associate SNPs with phenotypes and to estimate heritabilities. Bayesian method C0 (Habier et al. (2011)), i.e. allowing all SNPs to have non-zero effects (π = prior proportion of SNPs with zero effects = 0), was used to estimate marker-based heritabilities and the genetic and residual variances to be used in the association analysis. Bayes-Cπ (Habier et al. (2011)) was used to estimate π and then Bayes-B (Meuwissen et al. (2001)) was used for the association analyses. Missing genotypes were replaced with the mean genotype for that SNP. If the estimated π resulted in more SNPs fitted in the model than available degrees of freedom (df), a value of π that resulted in as many SNPs as available df was used (Serão et al. (2014)). The number of Markov chain Monte Carlo iterations used was 45,000, with 4,000 burn-ins. All SNP association analyses were performed using GenSel version 4.4 (Fernando and Garrick (2009)). GenSel provides estimates of the total genetic variance explained by the markers (TGVM) and of genetic variance explained by each non-overlapping 1-Mb SNP window across the genome (Wolc et al. (2012)). The 30 windows with greatest window variance were then investigated, and those within 2 Mb of each other were combined and reanalyzed. The final results included windows that explained at least 1% of the TGVM.

Models. Two models were evaluated in the GWAS for each trait, one with and one without the five candidate SNPs (WUR, MARC, ASGA, M1GA, and ALGA) as fixed effects. The CG was the only fixed-effect fitted in the model for all four traits. A linear contrast was used to estimate the effect of vaccination, since this effect is confounded with CG. Different covariates were used for each trait: weight at day 0 for ADG, S/P-d0, and S/P-Acc, and weight after acclimation for S/P-Par. The fixed effect estimates were obtained as the posterior mean and posterior standard deviation (PSD) of the 41,000 MCMCs. The posterior probability (PPr) of the estimate being greater or less then zero was used to assess significance (PPr > 0.9) of the fixed effects.

Results and Discussion

Although only 7% of the gilts were PRRSV+ at day 0 (S/P $\geq$ 0.4), all were PRRSV+ after acclimation, either from infection or vaccination (Table 1). Considering that gilts were exposed to PRRSV shortly after entry, the average acclimation period (40.1±14.0 days) suggested that S/P-Acc represented peak antibody response in these animals (Kim et al. (2011)).

Trait heritabilities and the effects of vaccination and candidate SNPs are presented in Table 2. Low heritability estimates (±PSD) were obtained for ADG, S/P-d0, and S/P-Par, with 0.09±0.04, 0.13±0.05, and 0.11±0.09, whereas S/P-Acc had a high heritability of 0.47±0.11. This high heritability agrees with Serão et al. (2014), who observed an estimate of 0.45±0.13 for PRRSV S/P ratio in sows after a PRRS outbreak. The heritability and PSD estimates for ADG and S/P-d0 indicate that traits most likely have a sizable heritable component, but the smaller sample size and larger variability observed for S/P-Par does not allow us to draw the same conclusion for this trait.

Table 2. Heritabilities and fixed effect estimates.

		Trait			
Effect[§]		ADG	S/P-d0	S/P-Acc	S/P-Par
Vaccination		0.04*	0.07*	-0.38*	0.23*
WUR	*Add*	0.01	0.02	0.02	0.10
	Dom	-0.02	-0.01	-0.11	0.16
MARC	*Add*	0.01	0.01	0.05	0.08
	Dom	-0.01	0.01	0.16*	-0.13*
ASGA	*Add*	0.03*	0.01	0.02	-0.08
	Dom	-0.02	-0.01	-0.12*	0.07
M1GA	*Add*	-0.01	0.02*	0.02	-0.20*
	Dom	0.01	-0.01	0.08*	0.02
ALGA	*Add*	0.03	0.01	-0.08*	-0.11
	Dom	0.03*	-0.01*	-0.10*	0.01
Heritability		0.09	0.13	0.47	0.11

ADG, average daily gain; S/P-d0, PRRSV ELISA sample-to-positive at day 0; S/P-Acc, PRRSV ELISA sample-to-positive after acclimation; S/P-Par, PRRSV ELISA sample-to-positive at first parity;
[§]Vaccination effect represents the vaccinated group;
The additive (Add) effect was calculated as the number of B alleles, whereas the dominance (Dom) effect was calculated as AB minus the average homozygotes;
*Estimates significant at posterior probability of being greater or less then zero of 0.9 (PPr > 0.9);

Vaccination against PRRSV had a significant (PPr>0.9) effect on all traits (Table 2). Herds that vaccinated had greater ADG (0.04±0.02 kg) and PRRSV antibody response at day 0 (0.07±0.01) and at parity 1 (0.23±0.01), than herds without PRRSV vaccination. In contrast, unvaccinated gilts had a much greater antibody response after acclimation (0.38±0.09) than vaccinated animals. These results suggest that vaccination slightly increases growth performance and reduces S/P during gilt acclimation. However, since the effect of vaccination was confounded with herd, these effects could potentially be an effect of better management of vaccinated herds in general; hence, conclusions should be taken with caution.

The effects and association of the five candidate SNPs are presented in Table 2. With the exception of WUR, all candidate SNPs were significantly associated (PPr>0.9) with at least two traits. This agrees with Serão et al. (2014), who also did not observe an association between the WUR genotype and S/P ratio, but contrasts to Boddicker et al. (2012), where WUR was associated with weight gain and viremia following experimental infection of nursery pigs with a specific type 2 PRRSV strain. The MARC SNP had

contrasting associations, showing positive and negative dominance effects for S/P-Acc (0.16±0.04) and S/P-Par (-0.13±0.08), respectively. The number of B alleles for ASGA was positively associated with ADG (0.03±0.01) but AB animals had lower S/P-Acc (-0.12±0.05) than homozygotes. The M1GA SNP was associated with all three S/P traits, showing a positive additive effect at day 0 (0.02±0.1) and a negative additive effect at parity 1 (-0.20±0.08), while it was dominant after acclimation (0.08±0.05). The dominance effect of ALGA was significant (PPr > 0.9) for ADG, S/P-d0, and S/P-Acc. In addition, S/P-Acc decreased with increasing number of B alleles (-0.08±0.04). All four candidate SNPs previously associated with S/P ratio (Serão et al. (2014)) were also associated with S/P-Acc in this study. Among the three S/P ratio traits used in this study, S/P-Acc was the most similar to that of Serão et al. (2014). These significant associations validate previous finds for PRRS antibody response.

The genomic regions associated with the traits evaluated in this study (%TGVM>1) are presented in Table 3. In general, the genomic regions associated with the traits did not differ whether the five candidate SNPs were included as a fixed effect or not in the GWAS models. Of the four traits analyzed, S/P-Par was the only trait without associations. For ADG, one region on SSC4 (77-81 Mb) accounted for over 1% of the TGVM. Interestingly, the WUR SNP is on SSC4 but 50 Mb downstream from the associated region. S/P-d0 had more regions associated (%TGVM>1) than any other trait in this study. A total of five QTLs, which accounted for over 50% TGVM, were found. Of the three regions with the largest effect, the SSC7 region at 130 Mb has been previously associated with PRRSV S/P ratio (Serão et al. (2014)), and the region on SSC2 (60 Mb) has been associated with interferon-gamma levels (Uddin et al. (2011)); little is know about the SSC6 region. For S/P-Acc, the SSC7 region (27-30 Mb) that was associated when the candidate markers were not fitted as a fixed effect, was not associated once the candidate SNPs were fitted. Two of the candidate markers (MARC and ASGA) are located within the MHC region, which includes this region. Therefore, when these SNPs are fitted in the model as fixed effects, they account for the TGVM that would have been accounted for by the SNP window, validating previous results (Serão et al. (2014)). The two regions on SSC13 that were associated with S/P-Acc have both been previously associated with *Mycoplasma hyopneumoniae* antibody titer (Uddin et al. (2011)).

Table 3. Genomic regions associated with average daily gain (ADG) and PRRSV antibody response.

Trait	Can. SNP	π	%TGVM	PPI	SSC	Mb
ADG	no	0.984	1.8	0.43	4	77-81
ADG	yes	0.984	1.0	0.37	4	80-81
S/P-d0	no	0.989	25.2	1	2	60
			16.0	0.99	7	130
			10.9	0.82	6	10
			4.2	0.42	18	30-33
			1.2	0.33	13	0
S/P-d0	yes	0.989	26.3	1	2	60
			15.3	0.97	7	130
			13.5	0.85	6	10
			3.4	0.44	18	27-33
			1.6	0.50	18	47
S/P-Acc.	no	0.991	1.3	0.44	7	27-30
			1.2	0.47	13	82-83
S/P-Acc.	yes	0.991	1.7	0.37	10	30-32
			1.4	0.39	13	82-83
			1.2	0.26	13	132-135

ADG, average daily gain; S/P-d0, PRRSV ELISA sample-to-positive at day 0; S/P-Acc, PRRSV ELISA sample-to-positive after acclimation;
Can. SNP, whether the five candidate SNPs (WUR, MARC, ASGA, M1GA, and ALGA) were fitted (yes) or not (no) in the GWAS model;
π, proportion of SNP with zero effects in the model;
%TGVM, percentage of the total genetic variance explained by the window; PPI, posterior probability of inclusion of the window.

Conclusions

The traits evaluated in this study had low (ADG, S/P-d0, and S/P-Par) or high (S/P-Acc) marker-based heritability estimates. Vaccination for PRRS increased ADG, and antibody response at day 0 and at parity 1, but led to lower antibody response just after acclimation. Candidate SNPs MARC, ASGA, M1GA, and ALGA were validated for PRRSV ELISA S/P ratio response, but the WUR SNP was not. Two regions on SSC7 previously associated with S/P ratio were further validated in this study. Interestingly, the MHC region was not associated once the candidate SNPs were included in the model as fixed effects, indicating that these were indeed capturing the TGVM. Therefore, these results suggest that variants in two regions on SSC7 may largely control PRRSV antibody response, measured as ELISA sample-to-positive ratio.

Acknowledgements

The authors appreciate the financial support of the Canadian Swine Health Board, Genome Canada, and PigGen Canada.

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