A WUR SNP is associated with European Porcine Reproductive and Respiratory Virus Syndrome resistance and growth performance in pigs

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Abstract

Porcine reproductive and respiratory syndrome (PRRS) causes decreased reproductive performance in breeding animals and increased respiratory problems in growing animals. The goals of the current study were 1) to examine whether individual variation applies to infection with PRRSV European strains and 2) to investigate the association of a single nucleotide polymorfism (SNP) WUR10000125 (WUR) at the interferon-inducible guanylate-binding protein 1 gene (*GBP1*) with average daily gain (ADG) in PRRSV infected and uninfected pigs. The experimental procedure consisted of two trials of 80 and 40 pigs that were infected with a wild-type or vaccinated with an attenuated European PRRS virus strain and monitored after infection or vaccination. Viral load and ADG were determined for each pig. In a third trial, the ADG for PRRSV-free pigs was monitored. All pigs were genotyped for WUR at the *GBP1* gene (AA and AG genotype were defined). Results indicated that there was individual variation in the viral load from pigs challenged with a wild-type or low virulent European PRRSV strain. Secondly, our data showed that WUR SNP was associated to ADG in vaccinated pigs. Thus, ADG in AG pigs was significantly higher than in AA ones after vaccinating with an attenuated PRRSV strain. However, the reverse happened in a PRRSV-free enviroment where the AA pigs were those that grew faster. Based on these results, there is a scope for selecting pigs according to their responses to PRRS virus infection with European strains.

Key words: Variation, piglets, PRRSV, genetic resistance, SNP.

1. 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). The biggest economic impact of PRRSV is reproductive failure in sows such as abortion, mummified and stillborn piglets and respiratory problems with reduced performance in growing animals (Zimmerman et al., 2006). Vaccination for protection against PRRSV is not fully successful (Murtaugh and Genzow., 2011; Geldhof et al., 2013), primarily because of the high degree of antigenic and genetic drift in viral structural and non-structural viral proteins and the capacity of the virus to subvert early innate immune responses (Fang et al., 2007; Mateu and Diaz., 2008). Methods other than vaccination must be explored to aid the control of this disease; one possibility is genetic improvement of the host to decrease the negative impact of this disease not only in the respiratory (mainly young pigs) but also in its reproductive form (associated to adult animals) (Lewis et al., 2007).

Since the discovery of PRRS virus, a body of evidence has emerged associating host genetics with different outcomes following PRRSV infection. Thus, Meishan and Large White pigs seem to be more resistant to the effects of the virus than Duroc and Pietrain pigs (Petry et al., 2005, 2007; Reiner et al., 2010). More recently, the heritability for viral load was estimated to be close to 0.3 after challenge with a North American PRRSV strain (Boddicker et al., 2012). This trait and the average daily gain (ADG) were associated with a single genomic region in chromosome 4 (SSC4) which is best represented by the SNP tag marker WUR1000125 (WUR), located in the 3' non-coding region of the interferon-inducible guanylate-binding protein 1 (*GBP1*) gene. Presently, the effect that this marker could have on the ADG in PRRSV-free pigs is unknown.

The information about genetic resistance to PRRSV has been generated by The PRRS Host Genetics Consortium using a PRRSV North American strain in commercial crossbred pigs (Lunney et al., 2011; Rowland et al., 2012). There is scarce of information about the genetic resistance to European PRRSV strains neither in the reproductive nor in the respiratory form of the disease

(Mathur et al., 2014). Threfefore, the objectives of the current study were (1) to examine whether individual variation to infection with European PRRSV strains exists and (2) to investigate the association of the WUR SNP at the *GBP1* gene with ADG in PRRSV infected and uninfected pigs.

2. Material and methods

Three complementary experiments were designed. In experiment 1, pigs were challenged with a wild-type PRRSV virus whereas, in Experiment 2, they were vaccinated with an attenuated PRRSV strain (PRRSV modified live vaccine). Finally, Experiment 3 was conceived to assess the effect of SNP WUR in PRRSV uninfected pigs.

2.1.- Experiment 1. Pigs challenged with wild-type PRRS virus

Eighty commercial Large White (LW) x Landrace (LS) female pigs were randomly selected at 11-12 weeks of age from high-health status farm located in the northern part of Spain. These pigs came from a negative PRRSV farm and they were porcine circovirus type 2 negative and clinically healthy at the beginning of the experiment. Pigs received non-medicated commercial feed *ad libitum* and had free access to drinking water. Animals were housed in an experimental farm (Vall Companys, Mequinenza, Huesca, Spain). Pigs were identified, ear-tagged and randomly distributed into pens of 10 animals assuring a stock density of 1 m^2 by animal and balanced by weight (range 25-30 Kg). After a 7-day acclimation period (day 0), pigs were experimentally infected intramuscularly with 10^5 (TCID50) of a wild-type European PRRSV strain responsible of clinical outbreaks in farms belonging to Vall Companys group. Blood samples were collected at 0, 7, 14, 21, 28, 35, 42, 56 and 70 days post-challenge (DPC).

2.2.- Experiment 2. Pigs vaccinated with an attenuated PRRSV strain (PRRSV modified live vaccine)

Forty commercial male pigs from LW x LS (26) and Duroc (14) genetic types were randomly chosen at 6-7 weeks of age from two high health status farms located in the Northern part of Spain. These pigs came from two negative PRRSV farms and they were porcine circovirus type 2 negative and clinically healthy at the beginning of the experiment. Pigs received non-medicated commercial feed *ad libitum* and had free access to drinking water. Animals were housed in an experimental farm (CEP, Torrelameu, Lleida, Spain) without mixing animals of different breeds. Pigs were identified, ear-tagged and randomly distributed into three pens, assuring a stock density of 1 m^2 by animal, and balanced by weight (range 10-20 kg). After a 7-day acclimation period (day 0), pigs were vaccinated intramuscularly with a commercial PRRS modified live vaccine as recommended by the manufacturer (Porcilis® PRRS, MSD Animal Health). Blood samples were collected at 0, 4, 7, 10, 14, 21, 28, 35, and 42 days post-vaccination (DPV). Body weight was collected at 0 and 42 DPV. Pigs were euthanized with an intravenous overdose of sodium pentobarbital at 42 DPV. At the time of slaughter, tonsils were collected, snap frozen in liquid nitrogen and stored at -80ºC until processed. The former (experiment 1) and this experimental protocol were approved by the Ethical Committee of the University of Lleida, with reference number (CEEA 01-05/13).

2.3. Experiment 3. Pigs not infected with PRRS virus

For this experiment, we used samples and data already available in our laboratory. Briefly, the data set included 617 Duroc and 53 LW x LS commercial pigs with data on live body weight at 80, 160, 180, and 205 days used for other studies (Estany et al., 2014). Pigs were from nine different batches raised in PRRSV negative farms.

2.4. PRRSV determination in serum and tonsil

PRRSV viremia was measured using a semi-quantitative TaqMan PCR assay for PRRSV RNA. The PCR was performed as a routine diagnostic test by personnel of the Group of

Saneajament Porci (GSP, Lleida, Spain). Briefly, total RNA was isolated from serum using the LSI™ MagVet™ Universal Isolation Kit (Thermo Fisher Scientific Inc) in accordance with the manufacturer's instructions. An internal positive control “IPC PRRS” was included within each sample and extracted according to manufacturing instruction to validate RNA extraction step. Samples were analyzed with LSI™ VetMAX™ PRRSV EU/NA Kit (Life Technologies, Thermo Fisher Scientific Inc). Viral RNA was amplified as a 1-step reverse transcriptase (RT)-PCR reaction, according to kit instructions. Each 25 µl-reaction contained 7 µL of RNA and 18 µL of Mix PRRS EU/NA from the kit. The RT-PCR reactions were carried out on a QST 7500 Real-Time PCR System (Life Technologies, Thermo Fisher Scientific Inc) in a 96-well format according to the manufacturer's recommendations (10 min at 45°C, 10 min at 95°C followed by 40 cycles of 15 sec denaturation at 95°C and 70 sec annealing at 60°C). For the construction of a standard curve, ranging from 10 to 10^6 copies/mL, serial dilutions of a template RNA were prepared and assayed along with the samples (provided in the LSI™ VetMAX™ PRRSV EU/NA Kit). The assay results were reported as the Log_{10} of PRRSV RNA copies/mL relative to the standard curve. Because of the sensitivity of PCR, less than 10 copies (before log-transformation) were assumed to have negligible amounts of virus in the serum relative to the standard and were given a value of 1 (corresponding to a log-transformed value of 0). Tonsil samples (50 mg, approximately) were grounded in pestle and mortar with LN_2 previous to RNA isolation with the TRI reagent (Sigma Aldrich) following the manufacturer’s instructions. A total of 2 µg of tonsil RNA was used in the RT-PCR reactions, as explained above.

2.5. DNA isolation of each animal

Genomic DNA was isolated from whole blood samples using standard protocols (Green et al., 2012). DNA quantity and purity was evaluated by a NanoDrop Spectrophotometer and quality was assured by sizing on agarose gels.

151

152 *2.6. Genotyping*

153 Animals were genotyped for the WUR molecular marker (SNPdb reference: rs80800372)
154 with a a real-time PCR-based custom allelic discrimiation assay (Life Technologies, Thermo Fisher
155 Scientific Inc). The assay was set up using the following primers and probes: WUR_F, AGA CCT
156 AGA ATC TCC ACA GAA TTT CCA; WUR_R, GGA AAG GAC AGT TCG CTT CTC TAG A;
157 WUR_A, VIC-CTG GGT GAT AAA TAA AT-NFQ; WUR_G, FAM-TGG GTG ATG AAT AAA T–
158 NFQ. Genotyping reactions were prepared in a final volume of 5 µl and contained 0.9 µM of each
159 primer, 0.2 µM of each probe, 1 x TaqMan Universal PCR Master Mix (Life Technologies, Thermo
160 Fisher Scientific Inc) and 10 ng of genomic DNA. Thermal cycling conditions consisted on an
161 initial holding step of 60 ºC x 1 min, followed by 40 cycles of 93 ºC for 5 sec and 60 ºC for 1 min
162 plus a final step of 60 ºC for 1 min.

163 Animals were genotyped as AA, AG and GG, which corresponds to the AA, AB, BB
164 nomenclature used in the literature (Boddicker et al., 2012). Because only few pigs were GG (Table
165 1), as in Boddicker et al., (2012, 2014[b]), the GG pigs were merged into the AG genotype for data
166 analysis.

167

168 *2.7.- Data analysis*

169 Viral load (VL) was quantified as the area under the curve (AUC) for log-transformed viremia
170 at 0, 4, 7, 10, 14, and 21 DPC or DPV in each pig having records on at least 5 time-points. AUC
171 was determined using a linear trapezoidal method with linear interpolation over the 21 days DPC or
172 DPV. Pigs that died before 21 DPC or DPV were removed from the analyses. In experiment 2, the
173 average daily gain (ADG) was calculated as the difference between BW at day 42 and day 0 divided
174 by 42. In Experiment 3, ADG for each given interval was calculated as the difference between BW
175 at the end and the beginning of the period divided by the difference of the final and the initial age.

Individual variability of response to PRRSV challenge with a wild-type PRRSV was assessed by the variance of VL within and across all genetic types. Moreover, contingency tables with the corresponding chi-square statistics were performed to study the putative association of the genetic type or the WUR genotype with the presence of PRRSV in tonsils. The effect of the SNP WUR on VL and ADG was estimated using a linear model including, in the most general case, the effects of the genetic type (DU, LW x LS), the WUR genotype (AA, AG) and the weight at beginning of the trial and then tested following an F-test. The effect of the batch was added for ADG in Experiment 3.

3. Results

3.1. Allele frequency of WUR polymorphism

The genotypic frequencies of WUR polymorphism is shown in Table 1 by experiment and genetic type. The allele A was the predominant one in all the genetic types included in this study, resulting in an overall frequency of 82.3%. Moreover, the allelic frequency of the allele A was very similar across genetic types, ranging from 75.0%, for Duroc in experiment 2, to 93.4%, for LW x LS in experiment 3.

3.2. Variation of VL in piglets challenged with wild-type PRRS virus

After challenging piglets with a wild-type PRRSV virus by intramuscular route, viremia peaked at 7 days post-challenge (DPC) and declined afterwards (Figure 1). At 21 DPC, some of them (6.3%) had already cleared the circulating virus, while others (65%) still had declining viremia levels until 28 or 35 DPC. Interestingly, PRRSV was detected in serum from pigs that had apparently cleared the virus previously at 21, 28 or 35 DPC (33.8%). These cases can be considered as a natural rebound of PRRSV in serum. All the pigs cleared the virus at 56 DPC. A wide range of

viral load was observed after challenging pigs with a wild-type PRRS virus (Table 2) with VL ranging from 32.2 to 104.5 Log_{10} of PRRSV RNA copies/mL*day. Finally, no significant association ($p>0.05$) was detected between the WUR genotype and VL after challenging with a wild-type virus (Table 2).

3.3. Variation of VL in piglets vaccinated with an attenuated PRRSV strain

After administering an attenuated PRRSV strain, PRRSV was detected in serum, at least once, in 22 out of 40 piglets (55%) without observing differences across genetic types (54 and 57% for LW x LD and Duroc pigs, respectively). Unfortunately, it was not feasible to determine VL as only 10% of the pigs had enough positive sample-points to calculate this trait. After finishing the experiment, PRRSV was detected in the tonsils of 13 out of 40 piglets (32.5%). No difference ($P>0.05$) was observed between genetic types (35% versus 29%, for LW x LS and Duroc, respectively) or WUR genotypes for the presence of PRRSV in tonsil after 42 post-vaccination (Table 3). PRRSV was not detected in tonsils from pigs which were negative in serum throughout the trial. However, the WUR genotype was associated to ADG in both genetic types during the first 42 DPV, with AG piglets performing better than the AA ones (Table 3).

3.4. Association of the WUR marker with ADG in PRRSV negative pigs

The AA pigs showed higher ADG than the AG pigs in PRRSV negative pigs both in Duroc and LW x LS genetic types throughout the rearing period but significant differences ($p<0.05$) were more consistently observed at the earliest stages of growth across genetic types (Table 4).

4.- Discussion and conclusion

In the current study, we provide evidence that there was variation in virus load in pigs challenged with a wild-type or attenuated European PRRSV strain across genetic types. Moreover,

226 we have shown that pigs carrying the allele G at the WUR SNP at the *GBP1* gene grow faster than
227 those which are homozygous for the allele A after vaccinating them with a attenuated European
228 PRRSV strain. The most striking result is that this effect is reversed in a PRRSV-free environment.

229 The observed variability in the virus load is consistent with the results reported in pigs
230 challenged with a wild-type North American strain (Boddicker et al., 2014[ab]). In line with these
231 results, we also observed a rebound effect, with positive pigs to PRRSV amongst those that have
232 apparently cleared the virus (Islam et al., 2013). However, we were unable to find an association
233 between VL and the WUR genotype after challenging pigs with a wild-type virus. This result
234 disagrees with previous findings with PRRSV North American strains (Boddicker et al, 2014[ab]).
235 However, the age at challenge can be critical. Thus, the pigs used in our trial were five weeks older
236 (12-week-old pigs) than those in the experiments using PRRSV North American strains (7-week-old
237 pigs). It must be taken into account that macrophages from young pigs are more permissive and
238 susceptible to PRRSV than those from older pigs (Thanawongnuwech et al., 1998). Thus, it could
239 be hypothezized that in terms of PRRSV suceptibility, the WUR marker is more influential in young
240 than in older pigs, in which other factors may also play a role in the resistance to the infection.
241 Further studies are required to test this hypothesis.

242 We have also observed variability in the virus load in pigs vaccinated with an attenuated
243 European PRRSV strain regardless of the pig genetic type. Moreover, a variation was also observed
244 in ADG in pigs vaccinated with attenuated PRRSV strains. This result reinforces that there is
245 variation in ADG after PRRSV challenge independently of the genotype (North American versus
246 European one) and the virulence of the PRRSV strain used (wild-type versus attenuated one)
247 (Boddicker et al., 2014[a,b]).

248 The presence of PRRSV was detected in the tonsils of 32.5% of the piglets after vaccinating
249 them with an attenuated European PRRSV strain but the virus has never been found in pig tonsils

from animals which were PRRSV negative in serum throughout the trial. This result suggests that a pig will not become a reservoir for PRRSV in its tonsil if it is not viraemic over 42 DPV with an attenuated strain. Moreover, PRRSV was only detected in the tonsils of 59% of the viraemic animals.

The WUR SNP on SSC4 has been associated with PRRSV resistance in growing pigs, with the AG challenged pigs having better performance (greater weight gain and lower PRRS viral load) that the AA pigs (Boddicker et al., 2012). Using an attenuated PRRSV strain, this result agrees with our findings. Moreover, for the first time in the literature, we have also assessed this association in a PRRS-free environment. In this scenario, we have found that ADG was higher for AA than for AG pigs, both in Duroc and LW x LS. Traditional selection in pigs is based on breeding values estimated from data collected in a few high-health status nucleus breeders. Because in this environment the allele A is more favourable, it is arguably to hypothesize that selection for rapid growth rate may have led to increase the frequency of the allele A at population level. However, in farms at risk of PRRSV, this advantage turns into disadvantage, since AA infected pigs can further boost the negative effects of PRRSV infection (Boddicker et al, 2014[ab]). This result stresses the need of accounting for genotype per environment interactions in the breeding programs.

In conclusion, there was individual variation in the virus load in challenged pigs with both wild-type and attenuated European PRRSV strain. Finally, our data indicated that a pig will not become a reservoir for PRRSV in its tonsil if it is not viraemic over 42 DPV with an attenuated PRRSV strain.

Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organization that could inappropriately influence or bias the content of the paper.

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Table 1.- Total number of pigs (genotypic frequency, %) and allelic frequency of WUR alleles (SNPdb reference: rs80800372) by experiment (1, 2 and 3) and genetic type.

	Genotype			Allelic frequency (%)	
Experiment	AA	AG	GG	A	G
1 LW x LS n=80	44 (55%)	36 (45%)	0 (0%)	77.5	22.5
2 LW x LS n=26*	17 (68%)	8 (32%)	0(0%)	84.0	16.0
2 Duroc N=14	8 (57%)	5 (35.8 %)	1 (7.1%)	75.0	25.0
3 LW x LS n=53	46 (86.8%)	7 (13.2%)	0(0%)	93.4	6.60
3 Duroc N=617	414 (67.1%)	185 (30%)	18 (2.9%)	82.2	17.8

LW x LS = Large white x Landrace breed
* It was not possible to genotype one animal

Table 2.- Least square means (±Standard Error, SE) for serum viral load by WUR genotype after challenging pigs with a wild-type PRRS virus (Experiment 1).

	WUR genotype		
Viral load (Log_{10} of PRRSV RNA copies/mL X day)	AA	AG	All the animals
Mean ±SE[1]	78.2+1.8[a]	77.2+2.3[a]	77.5+1.4
Range	51.8-104.5	32.2-97.2	32.2-104.5
Variance	146.9	192.4	163

[1] M means with different superscript differ significantly ($p<0.05$)

Table 3.- Least square means (±Standard Error, SE) for average daily gain and prevalence of PRRSV in tonsils at 42 DPV by WUR genotype and genetic type after vaccination with a PRRS modified life vaccine (Experiment 2).

	WUR genotype	
Trait	AA	AG
ADG LW x LS (g/day)		
Mean ±SE	825.7+34[a]	928.6+39[b]
Range	536.6-1036.6	731.7-1024.4
ADG Duroc (g/day)		
Mean ±SE	664.6+22[a]	702.4+42.1[b]
Range	585.4-792.7	573.2-817.1
Prevalence of PRRSV in tonsils (13 out of 40)		
LW x LS (9 out of 26)	5	4
Duroc (4 out of 14)	3	1

[1] [M] means with different superscript differ significantly (P<0.05)

Table 4. Least square means (±Standard Error, SE) for average daily gain (g/d) between different ages by genetic type and WUR genotype in PRRSV negative pigs (Experiment 3).

Breed	Age period (d)	Genotype		p-value
		AA	AG	
Duroc				
	80-160	762.58± 8.00	725.58±12.28	p<0.05
	160-180	764.80 ±12.11	725.98±18.60	p=0.08
	160-205	697.07±10.67	671.70±16.39	NS
LW x LS				
	80-180	842.28±12.00	733.96 ± 32.73	p<0.05
	80-205	836.82± 9.70	753.65±26.45	p<0.05

Figure caption list

Figure 1.- Representative patterns of serum viral load by day post-challenge (DPC) with a wild-type PRRS virus (experiment 1) by intramuscular route. Four pigs were included in depicting each pattern.