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Molecular markers associated with response to vaccination against Porcine Reproductive and Respiratory Syndrome virus in a commercial swine farm from southern Sonora

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Abstract

Porcine Reproductive and Respiratory Syndrome (PRRS) is a viral disease that seriously affects the swine industry. The main method of control is vaccination, which has shown variable effectiveness partly attributed to genetic variability in sows. The objective was to validate single-nucleotide polymorphisms (SNPs) associated with performance traits following PRRS vaccination in replacement sows. A population of 100 Landrace(¾) / Yorkshire(¼) commercial line sows of 6 months of age, an average weight of 108 kg, and negative for PRRS virus were used. After an acclimatization stage, sows were vaccinated against PRRS (day 0). Daily weight gain (DWG) and rectal temperature (RT) were recorded on days 0, 7, 14, 21, and 28. A blood sample was individually collected on day 40 and used for DNA extraction and genotyping of 59 SNPs. These SNPs were previously identified through genomic analysis as associated with a vaccination response indicator. The 59 SNPs were validated in this study using a statistical mixed-effects model that included DWG or RT as response variables. From 59 SNPs, 5 of them were associated with DWG ($P < 0.05$), and 3 with RT ($P < 0.05$). Interestingly, the SNPs in the genes DENND5A (rs81409931 and rs81216494) and COL22A1 (rs329119519 and rs81381462) were associated with both DWG and RT, and they showed a higher allele contribution effect on phenotypes. These 2 genes appeared to be functionally related to DWG; therefore, their corresponding 4 SNPs are proposed as genetic markers associated with the response to PRRS vaccination in replacement sows from southern Sonora.

Keywords: Genetic markers; PRRS; Sows; SNPs; Vaccination response.

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Vol. 10 | 2023

Study contribution

PRRS infection is a worldwide disease that seriously affects swine production. Vaccination is currently considered the main strategy to control PRRS infection. However, the highly variable response to vaccination has affected its efficacy and suggests a genetic basis associated with such a response. This study validated genomic SNPs as marker predictors for daily weight gain (DWG) and rectal temperature (RT) in sows vaccinated against the PRRS virus. Molecular marker analysis represents a valuable tool for the validation of polymorphisms as predictors for complex traits such as response to vaccination. In the current study, we detected four molecular markers from two candidate genes that appeared to be associated with muscle growth and function. This helps explain why these markers were associated with daily weight gain in vaccinated sows. Then, four genetic markers and two candidate genes are proposed as predictors for PRRS vaccination response in replacement sows.

Introduction

Swine production is currently managed under a technified system with animals maintained in one place for their entire production cycles, where the most relevant variables of the process are controlled, such as infrastructure, management, reproduction, genetics, and nutrition, among others, as well as environmental conditions of the place, including temperature and humidity. Health status also is central to the farm since it deals with the prevention and treatment of diseases existing in the region, as well as hygiene and biosecurity measures.[1] Swine diseases are a constant concern for producers. Sonora is considered a free area for certain swine diseases, for which there are effective prevention mechanisms, such as the implementation of vaccines and proper management. However, in the last decade, the swine industry has faced one of its biggest challenges, a viral disease called Porcine Respiratory and Reproductive Syndrome (PRRS) that affects pigs of all ages, mainly pregnant sows and piglets.[2]

PRRS disease is caused by a virus of RNA nature, a member of the family Arteriviridae, genus *Arterivirus*.[3] PRRS is currently considered one of the infectious diseases with the greatest economic and productive impact on the swine industry worldwide.[4] Holtkamp et al.[5] reported that the cost of total productivity losses due to the PRRS virus in US breeding and fattening herds was US 664 million annually. This disease is characterized by reproductive failure, reproductive tract infections in pregnant sows, growth retardation, and joint disease in young pigs. Economic and productive losses can range from 45–50 % and in their most severe form represents up to 90 % mortality in fetuses and newborns; breeding sows present abortions, dead fetuses, mummified fetuses, and respiratory diseases.[4]

Novel knowledge into the virology, evolution and host response to PRRS disease have expanded rapidly. However, new variants of the virus constantly emerge from outbreaks, which has seriously hampered the effectiveness of control strategies, including vaccination.[6] Therefore, PRRS control has been only partially effective despite major efforts to mitigate the adverse effects of this disease.[7]

PRRS virus has the particularity of having a high degree of mutagenesis, so there is a great genetic diversity of viral strains; this has caused the vaccines used to

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Vol. 10 | 2023

have low antigenicity, as well as a highly variable immune response after vaccination. The above has originated large economic losses worldwide due to increased mortality, deterioration of feed conversion rates, reduced daily weight gain, and an increase in the number of productive age sows that are discarded.[2] The first vaccine against the PRRS virus was applied two decades ago; however, the prevalence of the disease in swine herds remains high, so PRRS control through vaccination has been limited.[8]

Advances in molecular technologies have allowed the identification of candidate markers associated with complex phenotypes in pigs, such as response to vaccination and/or disease resistance.[7] Genome-wide associative studies (GWAS) are being used to detect genomic regions and SNP markers associated with PRRS virus vaccination.[9, 10] Candidate marker association studies have been proposed to validate genomic SNPs as genetic markers for vaccination response.[11] It demonstrates the potential of these technologies to decipher the genetic basis regulating the response to vaccination in sows and thus improve our understanding of the high genetic variability associated with this response.

Moreover, the study of breeds, using molecular techniques is critical and useful for their characterizing.[12, 13] Conservation of genetic diversity in animals requires the proper performance of conservation superiorities and sustainable handling plans that should be based on universal information on population structures, including genetic diversity resources among and between breeds.[14, 15] Genetic diversity is essential for genetic improvement, preserving populations, evolution, and adapting to diseases and variable environmental situations.[16, 17]

On the other hand, the determination of gene polymorphism is important in farm animal breeding,[18, 19] to define genotypes of animals and their associations with diseases and productive, reproductive, and economic traits.[20, 21, 22] SNP validation in animal populations appears to be a successful strategy to detect molecular markers associated with economically relevant phenotypic traits.[11] Therefore, the study validated previously detected genomic polymorphisms as genetic markers associated with daily weight gain and rectal temperature in response to PRRS virus vaccination in replacement sows.

Materials and methods

Ethical statement

All animal procedures performed in this study were revised and approved by the "Bioethics Committee from the Agronomic and Sciences Department (Permit 0031)", at the Instituto Tecnológico de Sonora (ITSON), México.

Experimental units

This study was conducted in a full-cycle commercial swine farm located in the Yaqui Valley, a subtropical region in northern México (LN: 27°28', LO: 109°59'), and included one hundred, 6-month-old replacement gilts of the Landrace(¾)/Yorkshire(¼) maternal line. All females were housed inside the quarantine area of the swine farm.

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Vol. 10 | 2023

Vaccination and PRRS monitoring

After an adaptation period of 7 days, a commercial vaccine against the PRRS virus was applied intra-muscularly, with the minimum immunizing dose proposed by the manufacturer (Modified active virus strain ATCC-VR-2332 of PRRS DICT50 propagated in cell cultures, Ingelvac PRRS MLV, Boehringer Ingelheim Laboratory). The day of vaccination was considered day 0 of the study.

Seven days before vaccination, blood samples were collected from each female and transported to the ITSON Animal Pathology Diagnostic Lab. Viral RNA was isolated from blood serum using the DNA/RNA Extraction TacoTM Kit (Thermo Fisher Scientist, USA), as well as an automatic nucleic acid extraction system based on magnetic separation technology, and following the manufacturer's instructions (GeneReach Biotechnology Corporation, Taiwan). With the RNA samples identified, real-time PCR for PRRS was performed using a commercial kit (Tetracore Nextgen Real-Time QT-PCR Target Specific reagents for the detection & differentiation of North American & European PRRSV Viral RNA), which recognizes a segment of ORF 7, reported as a number of PRRS virus RNA copies per milliliter of the sample (Cepheid Smart Cycler, Version 2.0). This molecular test was performed to ensure that all females included in the study had a negative diagnosis for the presence of the PRRS virus.

Phenotypic records collection

All females were weighed weekly using a livestock scale for medium-frame species to calculate daily weight gain (DWG; kg). Before going to the scale, rectal temperature (RT; ºC) was taken using a digital GLA M750 thermometer (GLA Electrónica Agrícolas). Measurements of both variables were collected on days 0, 7, 14, 21, and 28, concerning the day of application of the PRRS vaccine.

Indicator of response to vaccination (IRV)

A simple linear regression analysis was performed to estimate the IRV, which represents daily weight gain as a function of rectal temperature after PRRS vaccination. The regression coefficient ($\beta 1$), which indicates the change in daily weight gain per unit of change in rectal temperature, was considered the indicator of response to vaccination (IRV). Therefore, sows with a positive value of $\beta 1$ were classified as sows with a favorable response to vaccination, while those with a negative value of $\beta 1$ were considered non-favorable response sows.

Genomic SNP markers selection

In previous research work from our group, a genome-wide association study (GWAS) was performed to identify the genetic basis associated with the indicator of response to PRRS vaccination (IRV), as described by Luna-Nevárez et al.[23] In this study, the association of the genotypes of each SNP (i.e., 10,000) with the IRV was analyzed considering all SNPs simultaneously using the Bayesian method called Bayes C. Genomic windows that explained at least 1 % of the genetic variation were considered significant, and the SNPs included within them were retained to be used in an associative study.[24] Results of the study revealed a total of 59

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Vol. 10 | 2023

genomic SNPs as significant predictors of the IRV ($P < 0.0001$), and these SNPs were considered useful candidates for marker selection.

Candidate marker association study

Each SNP previously identified through whole genome analysis as predictor for the IRV ($n = 59$) was selected for a validation associative study between genotype and phenotype through the MIXED procedure.

The statistical model was:

$$y_{ijkl} = \mu + A_i + B_j + C_k \beta_1 + D_l + e_{ijkl}$$

The model included DWG or RT as response variables (y_{ijkl}), the population means (μ), fixed effects of SNP genotype (A_i) and dam's age (B_j), female initial weight as a covariate (C_k), β_1 as the coefficient of the fixed linear regressions for C, sire as random effect (D_l), and the residual effect (e_{ijkl}). Statistical significance was determined when P-value was less than 0.05 ($P < 0.05$). Descriptive statistics for DWG and RT were calculated using PROC MEANS. The assumption of normality and equality of variances was tested using the UNIVARIATE procedure.[25] Allele and genotypic frequencies, as well as deviation from Hardy-Weinberg equilibrium, were estimated through the ALLELE procedure.[26]

If the genotype term was found to be a significant source of variation ($P < 0.05$) in the associative analysis, the PDIFF option of the LSMEANS procedure was used to generate comparisons between means for each genotype, including Bonferroni adjustment. The allelic substitution effect (i.e., the effect of substituting one allele for another within the population) was calculated through a regression model including the allele term as a covariate.[27] Genetic effects of dominance and additivity for the favorable allele were estimated following the procedures described by Sherman et al.[28] All statistical analyses were performed using the statistical package SAS (Statistical Analysis System) version 9.4 (SAS Inst. Inc., Cary, NC), which includes the genetic analysis tools.

Results

Average values for the traits live weight (LW), daily weight gain (DWG), and rectal temperature (RT) are described in Table 1. DWG appeared to decrease between days 7 and 14, which coincided with an increase in RT. These results suggest that the main response to PRRS vaccination occurred 7-14 days later.

From the 59 SNPs previously reported as genomic predictors for the indicator of response to vaccination (IRV; Luna-Nevárez et al.)[23] in the current study only 8 of them resulted significantly associated with performing traits such as DWG and RT ($P < 0.05$). These SNPs were in the genes DENND5A (DENN Domain Containing 5), NDUFAF6 (NADH: Ubiquinone Oxidoreductase Complex Assembly Factor 6), ARL5B (ADP Ribosylation Factor Like GTPase 5B), ZNF143 (Zinc Finger Protein 143), and COL22A1 (Collagen Type XXII Alpha 1 Chain).

SNP identification and their corresponding genes, as well as allele and genotype frequencies for the 8 significant SNPs, are observed in Table 2. These 8 SNPs

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Vol. 10 | 2023

Table 1. Means and standard error of vaccination response variables across time

Traits[1]	Day 0	Day 7	Day 14	Day 21	Day 28
n	100	100	100	100	100
LW (kg)	109.0 ± 11.9	113.4 ± 11.5	116.6 ± 11.21	121.1 ± 11.41	125.9 ± 12.33
DWG (kg)	0.63 ± 0.07	0.46 ± 0.05	0.64 ± 0.06	0.69 ± 0.07	0.58 ± 0.09
RT (°C)	38.32 ± 0.89	38.55 ± 0.53	39.08 ± 0.38	38.91 ± 0.33	38.62 ± 0.75

[1]Live weight (LW); Daily weight gain (DWG); Rectal temperature (RT).

Table 2. Identification, gene location, and allele and genotype frequencies from the SNPs associated with DWG and RT in replacement sows

SNP ID[1]	Gen[2]	Allele frequency		Genotype frequencies		
		C	T	CC	CT	TT
rs81409931	DENND5A	0.36	0.64	0.13	0.46	0.41
rs80957569	NDUFAF6	0.64	0.36	0.41	0.46	0.13
		G	T	GG	GT	TT
rs81313701	ARL5B	0.80	0.20	0.64	0.32	0.04
		A	G	AA	AG	GG
rs81310106	ZNF143	0.77	0.23	0.59	0.36	0.05
rs329119519	COL22A1	0.20	0.80	0.04	0.32	0.64
rs81216494	DENND5A	0.28	0.72	0.08	0.40	0.52
rs80830201	Intergenic	0.49	0.51	0.24	0.50	0.26
rs81381462	COL22A1	0.75	0.25	0.56	0.38	0.06

[1] SNP ID = SNP Reference of the NCBI.
[2] Gene = Gene symbol (DENND5A = DENN Domain Containing 5; NDUFAF6 = NADH: Ubiquinone Oxidoreductase Complex Assembly Factor 6; ARL5B = ADP Ribosylation Factor Like GTPase 5B; ZNF143 = Zinc Finger Protein 143; COL22A1 = Collagen Type XXII Alpha 1 Chain).

were accomplished with minor allele frequency greater than 10 % (MAF > 0.10) and Hardy-Weinberg equilibrium test ($X^2 > 0.01$).

The least-square means ±standard error (SE) according to genotypes from the 8 significant SNPs are observed in Table 3. The SNPs associated with DWG were rs81409931 (P = 0.036), rs81313701 (P = 0.045), rs81310106 (P = 0.038), rs329119519 (P = 0.043), and rs80830201 (P = 0.049), whereas the SNPs associated with RT were rs80957569 (P = 0.018), rs81216494 (P = 0.031), and rs81381462 (P = 0.024). However, from these polymorphisms, only the SNPs rs81409931 and rs81216494 located within the gene DENND5A, and the SNPs rs329119519 and rs81381462 in the gene COL22A1, resulted as associated with both DWG and RT. The favorable genotypes were CC, AA, TT, and TT for the SNPs rs81409931, rs81216494, rs329119519, and rs81381462, respectively, because they showed the best favorable performance for DWG or RT.

The allele substitution effects for the 8 significant SNPs, as well as the additive and dominant fixed effects are observed in Table 4. Interestingly, the SNPs that showed a higher estimated effect for both DWG and RT were rs81409931 (P = 0.036) and rs81216494 (P = 0.031) in the gene DENND5A, as well as the SNPs rs329119519 (P = 0.045) and rs81381462 (P = 0.021) in the gene COL22A1. This suggested an important contribution of these genes and SNPs to the response of vaccination in sows measured by the performance traits DWG and RT. Additionally, an additive genetic effect (P < 0.05) was confirmed for the favorable allele of these 4 SNPs.

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Vol. 10 | 2023

Table 3. Least square means ± SE according to SNP genotypes for the traits DWG and RT in replacement sows

SNP ID[1]	Trait[2]	N	Least square means by SNP genotype ± SE			P-value
			CC	CT	TT	34
rs81409931	DWG	100	0.53 ± 0.10[a]	0.51 ± 0.09[a]	0.34 ± 0.05[b]	0.036
rs80957569	RT	100	38.21 ± 0.06[a] GG	39.06 ± 0.05[b] GT	38.90 ± 0.10[b] TT	0.018
rs81313701	DWG	100	0.48 ± 0.03[a] AA	0.44 ± 0.04[a] AG	0.28 ± 0.14[b] GG	0.045
rs81310106	DWG	100	0.41 ± 0.04[a]	0.36 ± 0.04[a]	0.54 ± 0.14[b]	0.038
rs329119519	DWG	100	0.27 ± 0.14[a]	0.45 ± 0.10[b]	0.48 ± 0.03[b]	0.043
rs81216494	RT	100	38.48 ± 0.08[a]	38.83 ± 0.06[b]	39.09 ± 0.07[b]	0.031
rs80830201	DWG	100	0.32 ± 0.06[a]	0.50 ± 0.04[b]	0.48 ± 0.05[b]	0.049
rs81381462	RT	100	39.03 ± 0.05[a]	38.79 ± 0.06[a]	38.11 ± 0.06[b]	0.024

[1] SNP ID = SNP Reference of the NCBI.
[2] Daily weight gain (DWG); Rectal temperature (RT).
[a, b] Means with different superscripts in the same row are significantly different ($P < 0.05$).

Table 4. Allele substitution effect estimates and fixed effects of the additive and dominance of the favorable allele for the traits DWG and RT in replacement sows

SNP ID[1]	FA[2]	Allele substitution effects			Fixed effects	
		P-value[3]	Estimate[4]	SE	Additive[5]	Dominant[6]
rs81409931	C	0.036	0.124	0.05	0.095	0.075
rs80957569	C	0.018	0.105	0.06	0.034	0.505
rs81313701	G	0.040	0.102	0.05	0.100	0.060
rs81310106	G	0.041	0.090	0.04	0.065	0.115
rs329119519	G	0.045	0.149	0.05	0.105	0.075
rs81216494	A	0.031	0.171	0.05	0.305	0.045
rs80830201	G	0.047	0.117	0.06	0.080	0.100
rs81381462	T	0.021	0.146	0.07	0.460	0.220

[1] SNP ID = SNP Reference of the NCBI.
[2] Favorable allele (FA) = Allele showing a favorable effect on phenotype.
[3] P–values obtained from allele substitution analysis in SAS, which included the term genotype as covariate (significance is determined by $P < 0.05$).
[4] Estimates of the effect expressed in units of the traits.
[5] Additive effect was estimated as the difference between the 2 homozygous means divided by 2.
[6] Dominant effect was calculated as the deviation of the heterozygous from the mean of the 2 homozygous.

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Vol. 10 | 2023

Discussion

Infectious animal diseases are one of the main concerns of producers, health authorities, and international markets. This is due to the capacity of diseases to spread through the movement of live animals, their products and by-products, the movement of people, as well as the asymmetries in the implementation of biosecurity measures and good practices along the production chain.[29] Porcine Reproductive and Respiratory Syndrome (PRRS) virus has caused significant economic losses in the swine industry worldwide. Control of the PRRS virus relies on aspects such as early diagnosis and monitoring, biosecurity, herd management, and immunization.[30] Vaccination against the PRRS virus is currently considered the main strategy to prevent and control the PRRS infection. However, the highly variable response to vaccination has affected its efficacy and suggests a genetic basis associated with such a response.

Genomic studies performed in pigs and sows infected by the PRRS virus provided evidence that several candidate SNP markers appeared to be involved in response to PRRS infection. These results strongly suggest a genetic basis underlying tolerance/resistance to PRRS infection, which could also explain the highly variable response to vaccination.[11, 31] However, genomic SNP should be tested in external populations through an associative study to be validated as genetic markers.[32] Molecular marker validation represents a valuable tool for the discovery of genomic polymorphic variants (SNPs) that are associated with economically important traits in pigs, including the vaccination response to economically important diseases such as PRRS.[33]

The current study included a total of 59 SNPs that were identified in a previous genomic study performed by our research group,[11] These genomic SNPs explained about 32 % of the genetic variance associated with an indicator of response to vaccination (IRV). The IRV was calculated using a simple linear regression model including DWG as the independent variable (x) and RT as the dependent variable (y). The 59 SNPs were validated through an associative study between genotypes with DWG and RT variables, resulting in 8 SNPs as predictors of the traits under study. Both DWG and RT traits are extremely important phenotypic variables due to their close relationship with pig body development and growth.[23]

A more detailed study of these markers identified 2 SNPs associated with DWG (rs81409931and rs329119519) and 2 SNPs associated with RT (rs81216494 and rs81381462) in a population of quarantined sows following PRRS virus vaccination. The SNPs rs81409931 and rs81216494 are located within the gene DENND5A (DENN Domain Containing 5 A), and the SNPs rs329119519 and rs81381462 are located in the gene COL22A1 (Collagen Type XXII Alpha 1 Chain).

The DENND5A gene codes for a protein that catalyzes the conversion of guanosine diphosphate (GDP) to guanosine triphosphate (GTP), thereby converting inactive GDP-bound Rab proteins to their active form in GTP. Once active, GTP participates in the gluconeogenesis cycle and the conversion of pyruvate to phosphoenolpyruvate. At this point, two high-energy molecules, ATP and GTP, have been used. Continuing the cycle, the biochemical reactions give glucose as the final product of gluconeogenesis, which passes through the bloodstream to the tissues, including muscle.[34]

Skeletal muscles are critical to the regulation of glucose metabolism. During the time between feeding and short-term fasting, the blood glucose remains within normal limits, partially due to the ability of the muscles to provide energy substrates

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Vol. 10 | 2023

for the liver (i.e., lactate, pyruvate, and alanine), which can be converted into glucose. This is to maintain energy reserves at optimal levels so that the organism can continue with its normal physiological activities, such as muscle growth and development.[35] This indicates that the DENND5A gene is related to muscle growth, which helps to explain its direct association with daily weight gain in this study.

The COL22A1 gene encodes for a member of the collagen family that contributes to the stabilization of myotendinous junctions and strengthens skeletal muscle junctions during contractile activity. The encoded protein is deposited in the basement membrane zone of the myotendinous junction, which is present only in the tissue junctions of muscles, tendons, heart, articular cartilage, and skin. COL22A1 protein is part of the basement membranes and tissue attachment. Skeletal muscles are composed of bundles of muscle fibers, which envelop the connective tissue. Its main component is the muscle cell or fiber; however, there are other types of cells included in the muscle, such as vascular, connective, adipose, and nervous tissue cells that are predominantly constituted by collagen.[36] Morón-Fuenmayor et al.[37] studied the *Longissimus thoracis*, *Latisimus dorsi*, and *Semitendinosus* muscles of commercial crossbred bulls to determine the concentration of total collagen and its fractions (soluble and insoluble). As a result, they obtained that the *Longissimus thoracis* had the highest percentage of soluble collagen ($P < 0.05$) concerning the *Latissimus dorsi* and *Semitendinosus* by 6 and 12 % points, respectively.

The muscle is composed of contractile and non-contractile structures. Muscle fibers represent the major part of the muscle and are responsible for force generation. Non-contractile muscular components such as the perimysium, epimysium, and endomysium structure and organize the muscle while transmitting the force generated by tendons and bones. García et al.[38] demonstrated that there is a relationship between the non-contractile components and tenacity, which represents the capacity of the muscle to absorb energy. Lebret and Guillard[39] considered that glycolytic fibers present a greater pH drop than oxidative fibers due to their higher glycogen and lactic acid content, although they concluded that this relationship is not always observed. Another study evaluated muscle structure and concluded that the amount of collagen present in the muscle fiber is linked with the availability of metabolites, specifically glycogen.[40] Therefore, these results suggested that the COL22A1 gene is linked to the adequate absorption of energy by the muscle for its function, development, and growth. This appeared to explain its relationship with daily weight gain in the current study.

Conclusions

The application of molecular technologies appeared to be an effective strategy to identify genetic markers associated with complex traits in sows. In this study, four genomic SNPs within the genes DENND5A and COL22A1 were successfully validated as predictors for daily weight gain and rectal temperature in sows vaccinated against the PRRS virus. After statistical associative validation, these molecular markers are useful for designing genetic selection programs. Then, we propose four SNPs for being included in marker-assisted selection programs to improve response to PRRS vaccination in sows.

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Vol. 10 | 2023

Data availability

Data will be made available upon reasonable request.

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Conflicts of interest

The authors have no conflict of interest to declare regarding this publication.

Author contributions

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