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Abstract
Profits for commercial pork producers vary in part because of sow productivity or sow productive life (SPL) and replacement costs. During the last decade, culling rates of sows have increased to more than 50% in the United States. Both SPL and culling rates are influenced by genetic and nongenetic factors. A whole-genome association study was conducted for pig lifetime reproductive traits, including lifetime total number born (LTNB), lifetime number born alive (LNBA), removal parity, and the ratio between lifetime nonproductive days and herd life. The proportion of phenotypic variance explained by markers was 0.15 for LTNB and LNBA, 0.12 for removal parity, and 0.06 for the ratio between lifetime nonproductive days and herd life. Several informative QTL regions (e.g., 14 QTL regions for LTNB) and genes within the regions (e.g., *SLC22A18* on SSC2 for LTNB) were associated with lifetime reproductive traits in this study. Genes associated with LTNB and LNBA were similar, reflecting the high genetic correlation (0.99 ± 0.003) between these traits. Functional annotation revealed that many genes at the associated regions are expressed in reproductive tissues. For instance, the *SLC22A18* gene on SSC2 associated with LTNB has been shown to be expressed in the placenta of mice. Many of the QTL regions showing associations coincided with previously identified QTL for fat deposition. This reinforces the role of fat regulation for lifetime reproductive traits. Overall, this whole-genome association study provides a list of genomic locations and markers associated with pig lifetime reproductive traits that could be considered for SPL in future studies.

Keywords
ratio between lifetime nonproductive days and herd life, removal parity, sow lifetime litter size, whole-genome association

Comments
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Whole-genome association analyses for lifetime reproductive traits in the pig

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ABSTRACT: Profits for commercial pork producers vary in part because of sow productivity or sow productive life (SPL) and replacement costs. During the last decade, culling rates of sows have increased to more than 50% in the United States. Both SPL and culling rates are influenced by genetic and nongenetic factors. A whole-genome association study was conducted for pig lifetime reproductive traits, including lifetime total number born (LTNB), lifetime number born alive (LNBA), removal parity, and the ratio between lifetime nonproductive days and herd life. The proportion of phenotypic variance explained by markers was 0.15 for LTNB and LNBA, 0.12 for removal parity, and 0.06 for the ratio between lifetime nonproductive days and herd life. Several informative QTL regions (e.g., 14 QTL regions for LTNB) and genes within the regions (e.g., *SLC22A18* on SSC2 for LTNB) were associated with lifetime reproductive traits in this study. Genes associated with LTNB and LNBA were similar, reflecting the high genetic correlation (0.99 ± 0.003) between these traits. Functional annotation revealed that many genes at the associated regions are expressed in reproductive tissues. For instance, the *SLC22A18* gene on SSC2 associated with LTNB has been shown to be expressed in the placenta of mice. Many of the QTL regions showing associations coincided with previously identified QTL for fat deposition. This reinforces the role of fat regulation for lifetime reproductive traits. Overall, this whole-genome association study provides a list of genomic locations and markers associated with pig lifetime reproductive traits that could be considered for SPL in future studies.

Key words: ratio between lifetime nonproductive days and herd life, removal parity, sow lifetime litter size, whole-genome association

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INTRODUCTION

Improving sow productive life (**SPL**) would greatly affect profits of commercial pig farms. However, culling rates of sows have been increasing in many countries. In the United States, the culling rate increased from 44.6 to 51.0% between 2000 and 2007 (http://www.thepigsite.com). Increased culling has led to greater replacement rates, which decreases economic efficiency for commercial pig farms. Hence, improving SPL by reducing the culling rate would contribute to improving commercial pig farm production efficiency.

Sow productive life can be measured by various methods (Stalder et al., 2004), and these include length of productive life, culling rate, lifetime prolificacy, average parity at removal, and lifetime nonproductive days. Many genetic and nongenetic factors influence SPL. Heritability estimates for lifetime reproductive traits are generally low (0.09 to 0.17; Serenius and Stalder, 2004; Nikkilä et al., 2010). An association study using candidate genes that are involved in longevity pathways of model organisms reported that genetic markers need to be considered for marker-assisted selection programs for improvement of SPL (Mote et al., 2009).

The development of the Illumina PorcineSNP60 BeadChip (Ramos et al., 2009) via the efforts of the International Swine Genome Sequencing Consortium (http://piggenome.org/) and the availability of new statistical tools (GenSel software at http://bigs.ansci.iastate.edu) based on Bayesian statistics have allowed researchers to conduct whole-genome association studies (**WGAS**) on many traits in the pig. Therefore, the objective of the present research was to conduct a WGAS for identifying genetic markers associated with several SPL traits, including lifetime total number born (**LTNB**), lifetime number born alive (**LNBA**), removal parity, and the

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ratio between lifetime nonproductive days and herd life (**LNPDR**).

MATERIALS AND METHODS

Animal care guidelines were followed according to the Institutional Animal Care and Use Committee at Iowa State University.

Animals and Phenotypes

A total of 818 gilts from commercially available maternal genetic lines were included in the study. The sows were housed on a single farm that used breeding stock from Newsham Choice Genetics (West Des Moines, IA). These animals belonged to either a Large White grandparent maternal line or a Large White × Landrace parent maternal line and were used for an earlier candidate gene study (Fan et al., 2009). The traits included LTNB, LNBA, removal parity, and LNPDR, and were recorded to a maximum of 9 parities on the animals during the years 2005 to 2009. The traits LTNB and LNBA contained data from parity 1 to the removal parity (or last parity in the case of the small number of sows still remaining in the herd after parity 9). The number of lifetime nonproductive days was considered the number of days from first service to conception (or to removal if no litter was produced) plus the weaning to conception interval in each parity (or weaning to removal interval). For animals without a record of first service, the number of days from herd entry to removal was considered the lifetime nonproductive days. The herd life was the difference in days between the herd entry date and removal date.

Because the animals were culled at different parities and because of the occasional lack of data for some animals, the number of animals considered in analyses was different for each of the traits studied. The phenotype statistics, such as mean and SE, were calculated with the R software (http://www.r-project.org). The genetic correlation between LTNB and LNBA was analyzed by ASREML using a bivariate animal model with pedigree-based relationships to infer covariation between animals and fixed effects including genetic line and cohort group based on animal entry into the farm.

DNA Isolation, SNP Array Genotyping, and Quality Control

An overview of genomic DNA isolation and quantification has been reported in an earlier publication (Fan et al., 2009). Genomic DNA samples with an amount of 700 to 1,000 ng, a ratio of $A_{260/280}$ (where A is absorbance, nm) between 1.50 and 1.90, and a concentration greater than 20 ng/μL were used for the PorcineSNP60 BeadChip genotyping. Genotyping was performed commercially at GeneSeek Inc. (Lincoln, NE). The SNP with call rates ≤80%, Gentrain scores ≤40%, minor allele frequencies ≤0.001, and *P*-values <0.0001 for a χ^2 test for Hardy-Weinberg equilibrium were excluded from the data set. After these quality control measures, a total of 57,814 SNP from 64,232 SNP were qualified for association analyses.

Population Stratification Analysis

The animals in the study were from 2 genetic lines but originated from Large White or Large White × Landrace interbreed crossing. Population stratification was examined using an identical-by-state distance clustering method in PLINK program (Purcell et al., 2007). To account for the limited line differences in phenotype, line was included as a fixed effect in later analyses, and this reduced the possibility that markers could pick up population stratification effects.

Genome-Wide Association Analyses

The analyses were implemented separately for each trait with the Bayes C model averaging approach described by Kizilkaya et al. (2010) using GenSel software (http://bigs.ansci.iastate.edu). The following statistical model was used:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Zu} + \mathbf{e},$$

where $\mathbf{y}$ is the vector of phenotypes, $\mathbf{X}$ is an incidence matrix of fixed effects ($\boldsymbol{\beta}$), $\mathbf{Z}$ is a matrix of SNP genotypes that were fitted as random effects ($\mathbf{u}$), and $\mathbf{e}$ is the vector of random residual effects assumed to be normally distributed, $N(0, \sigma_e^2)$. The fixed factors used in this statistical model were gilt line and cohort group based on animal entry date on farm. Individual SNP effects were estimated from a mixed model with a probability of 0.995 that any SNP would have a zero effect such that approximately 250 to 300 nonzero SNP effects were fitted per iteration of each Markov chain. This probability (0.995) was selected on the assumption that 250 to 300 SNP markers (0.5% of 57,814 SNP markers) may explain the variation in the sow lifetime reproductive traits. The probability 0.995 was estimated from our previous reproductive trait analyses (unpublished data). A total of 50,000 Markov iterations, with a burn-in of 1,000 iterations, were run for the analyses. The results from these analyses included posterior distributions for the effects of each of 57,814 markers, adjusted for the portfolio of all the other fitted marker effects in the model, which changed in each iteration of the chain.

The effect of any particular QTL may be distributed across numerous SNP in linkage disequilibrium with the QTL, resulting in individual SNP effects that tended to underestimate the real QTL effect. Accordingly, the posterior means of the SNP effects were collectively used to predict the genomic merit of sliding chromosomal regions, including 5 consecutive SNP based on the physical map order. The variation in genomic merit for this chromosome fragment across the population of

animals was expressed as a proportion of variance in whole-genomic prediction of merit to identify the most informative genomic regions. There are 11,563 unique SNP windows with 5 consecutive SNP in the whole genome. Therefore, the expected proportion of variance accounted for by 1 window was 8.6×10^{-5} (1/11,563). The estimated proportion of genetic variance contributed by sliding windows of consecutive SNP was plotted against genomic marker locations using R software. The genomic locations or SNP windows with the greatest contributions were considered the most likely regions to be associated with the trait and were defined as the QTL. A portfolio of 12 to 22 QTL was so identified for each trait.

Bootstrap Analysis for Hypothesis Testing

Bootstrap samples were produced using the posterior means of the 57,814 SNP to construct the distribution of the test statistic (genetic variance of a SNP window) for each putative QTL. This involved creating 1,000 bootstrap data sets for each trait. A bootstrap sample $\mathbf{y_j}$ for replicate j was created using the posterior means of the fixed $\hat{\boldsymbol{\beta}}$ and SNP $\hat{u}_i$ effects, except that all those SNP contained in the window that formed the QTL were excluded, and a vector of simulated residuals was added, formed by sampling a vector of independent standard normal deviations, $\boldsymbol{\varepsilon}_\mathbf{j}$, one deviation for each animal, scaled by the posterior mean of the residual SD, $\hat{\sigma}_e$, according to the following equation with $\mathbf{z_i}$ the ith column of $\mathbf{Z}$, with $\mathbf{X}$ and $\mathbf{Z}$ defined as previously:

$$\mathbf{y_j} = \mathbf{X}\hat{\boldsymbol{\beta}} + \sum_{i=1, i \notin QTL}^{i=57,814} \mathbf{z_i}\hat{u}_i + \hat{\sigma}_e \boldsymbol{\varepsilon}_\mathbf{j}.$$

These bootstrap samples were constructed according to the null hypothesis of no QTL in the identified SNP window. Each bootstrap sample was reanalyzed using the Bayes C model used for the real data, and the genetic variances of the SNP window corresponding to the QTL were accumulated for comparison with the test statistic represented by the genetic variance of the SNP window identified in the analysis of the real data. If just 1 bootstrap statistic from the 1,000 simulated exceeded the test statistic from the real data, the comparison-wise P-value was determined to be $0.001 < P < 0.002$. Only QTL with $P < 0.01$ were considered for gene searching and functional annotation. Multiple testing was taken into account using the probability of false positives as in Fernando et al. (2004). That approach controls the probability of false positive conclusions across all the tests undertaken, rather than the probability of making 1 mistake over all tests, as would be the interpretation of an experiment-wise error correction.

Gene Search and Functional Annotation

Gene searches were carried out in the highly associated ($P < 0.01$) QTL using the *Sus scrofa* 9 genome build (http://uswest.ensembl.org/Sus_scrofa/Info/Index), and HUGO Gene Nomenclature Committee nomenclature was used in the manuscript for gene symbols. The genomic sequences of associated gene-poor chromosomal regions were aligned against the human genome through National Center for Biotechnology Information nucleotide Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The human genes within the homologous sequences (E value $<1 \times 10^{-9}$) were considered to be present in the associated gene-poor pig chromosomal regions. Functional annotation for these genes was performed based on gene ontology using Database for Annotation, Visualization and Integrated Discovery software (http://david.abcc.ncifcrf.gov). Previously identified QTL were evaluated at associated chromosomal regions using the PigQTLdb (http://www.animalgenome.org/cgi-bin/gbrowse/pig/) for each trait.

RESULTS

Population Stratification Analysis

A population stratification analysis based on the SNP genotypes using identical-by-state distance clustering showed that gilts from the 2 lines were classified into 1 population. This suggests that no significant genetic difference existed between these 2 lines. However, pedigree information demonstrated that there were more relationships within than between lines, so line was included in the model to avoid line differences contributing to associations.

Association Analyses for Lifetime Reproductive Traits

The phenotypic means (±SE) of LTNB, LNBA, removal parity, and LNPDR were 42.0 ± 1.24 piglets, 38.37 ± 1.13 piglets, 2.95 ± 0.09 parities, and 0.27 ± 0.01, respectively, across 9 parities. The proportion of phenotypic variance explained by markers was 0.15 for LTNB and LNBA, 0.12 for removal parity, and 0.06 for LNPDR (Table 1). Several QTL (12 to 19) were associated ($P < 0.01$) with lifetime reproductive traits [Table 2, Supplemental Table 1 (http://jas.fass.org/content/vol89/issue4/), and Figure 1A to 1C]. The details for all associated QTL regions, genes within the regions, the association P-values by bootstrap analyses, and previously reported QTL in these regions are presented in Supplemental Table 1 for all traits. Most of the associated regions in this study were at previously reported QTL regions related to reproductive and fat traits in pig genome build 9 (Table 2 and Supplemental Table 1; http://www.animalgenome.org/cgi-bin/gbrowse/pig/).

Table 1. Posterior means of variance components explained by whole-genome SNP markers for lifetime reproductive traits in a study using maternal pig lines

Trait[1]	No. of animals	Genetic variance	Residual variance	Estimated total variance	Proportion of phenotypic variance explained by markers
LTNB	813	186.57	1,040.60	1,227.17	0.15
LNBA	818	155.11	872.46	1,027.56	0.15
Removal parity	718	0.715	5.28	6.00	0.12
LNPDR	718	0.005	0.08	0.085	0.06

[1]The traits were recorded to a maximum of 9 parities. LTNB = lifetime total number born; LNBA = lifetime number born alive; LNPDR = ratio between lifetime nonproductive days and herd life.

The significantly associated ($P < 0.01$) QTL regions and genes within the regions were similar for LTNB and LNBA. The concordance of QTL regions for the 2 traits is not surprising given the very high genetic correlation (0.99 ± 0.003) between them. A total of 14 QTL regions were associated with LTNB and LNBA. Among those, QTL regions on SSC2 (position 0.2 to 0.8 Mb in the *Sus scrofa* 9 genome build) and on SSC9 (position 6.98 to 7.51 Mb in the *S. scrofa* 9 genome build) were very highly significantly ($P < 0.001$) associated with both LTNB and LNBA (Table 2, Supplemental Table 1, and Figure 1; because of the near perfect genetic correlation between LTNB and LNBA, only the illustration of LTNB is presented). These QTL regions are contributing almost 4 to 5 times more than the expected proportion of variance accounted for by 1 window (8.6×10^{-5}; Figure 1). Functional annotation using the Database for Annotation, Visualization and Integrated Discovery software revealed that 11 genes from a total of 20 genes in these regions are expressed in reproductive tissues. For example, the gene *SLC22A18* at a QTL region on SSC2 (Supplemental Table 1) is known to play a role in reduced fetal intrauterine growth (Salas et al., 2004).

A QTL region on SSC5 (positions 37.44 to 38.25 Mb in the *S. scrofa* 9 genome build) containing the potassium voltage-gated channel subfamily C, member 2 (*KCNC2*) gene was very highly significantly ($P < 0.001$) associated with removal parity among the 19 associated ($P < 0.01$) QTL regions (Table 2, Supplemental Table 1, and Figure 1B). The proportion of genetic variance for this region is 3 times larger than the expected proportion of variance accounted for by 1 window (8.6×10^{-5}; Figure 1B). A total of 12 QTL were very significantly ($P < 0.01$) associated with LNPDR. Among them, a QTL at SSC14 (position 39.16 to 40.71 Mb in the *S. scrofa* 9 genome build), which was contributing 1.5 times the expected proportion of variance accounted for by 1 window, was very highly significantly ($P < 0.001$) associated with LNPDR (Table 2, Supplemental Table 1, and Figure 1C). This region contains 10 reproduction-related genes (Supplemental Table 1), including tumor necrosis factor receptor-associated factor (TRAF)-type zinc finger domain-containing protein 1 (*TRAFD1*) and sirtuin (silent mating type information regulation 2 homolog) 4 (*SIRT4*), which are involved in fertilization and insulin secretion, respectively.

DISCUSSION

In the present study, a WGAS using the PorcineSNP60 BeadChip was performed using Bayes C model averaging with random SNP effects for pig lifetime reproductive traits, including LTNB, LNBA, removal parity, and LNPDR, recorded to a maximum of 9 parities in com-

Table 2. The summary of significantly ($P < 0.01$) associated QTL regions and some important genes within the regions for lifetime reproductive traits in maternal pig lines

Trait[1]	No. of associated regions	SSC for associated regions	Some important genes at the associated regions (SSC)[2]	Previously identified QTL at the highly associated regions (SSC)[3]
LTNB	14	1, 2, 3, 5, 7, 9, 13, 14, 17	*FUT9* (1), *SLC22A18* (2),[4] *P2RY6* (9)[4]	Mummified pigs (2)
LNBA	14	1, 2, 3, 5, 7, 9, 13, 14	*FUT9* (1), *SLC22A18* (2),[4] *P2RY6* (9)[4]	Mummified pigs (2)
Removal parity	19	1, 2, 3, 5, 8, 9, 11, 12, 13, 14, 16, 18	*FUT9* (1), *KCNC2* (5)[4]	Right teat number, fat, and haptoglobin concentration (5)
LNPDR	12	5, 8, 9, 12, 14, 17, X	*TRAFD1* (14),[4] *FGF2* (8)	Fat, leg (hind) score, and haptoglobin concentration

[1]The traits were recorded to a maximum of 9 parities. LTNB = lifetime total number born; LNBA = lifetime number born alive; LNPDR = ratio between lifetime nonproductive days and herd life.

[2]*FUT9* = fucosyltransferase 9 [α(1,3)fucosyltransferase]; *FGF2* = fibroblast growth factor 2; *KCNC2* = potassium voltage-gated channel subfamily C, member 2; *P2RY6* = P2Y purinoceptor 6; *SLC22A18* = solute carrier family 22, member 18; *TRAFD1* = tumor necrosis factor receptor-associated factor (TRAF)-type zinc finger domain-containing protein 1.

[3]The QTL information was obtained from PigQTLdb (http://www.animalgenome.org/cgi-bin/gbrowse/pig/).

[4]Indicates genes at highly associated regions and their chromosomes.

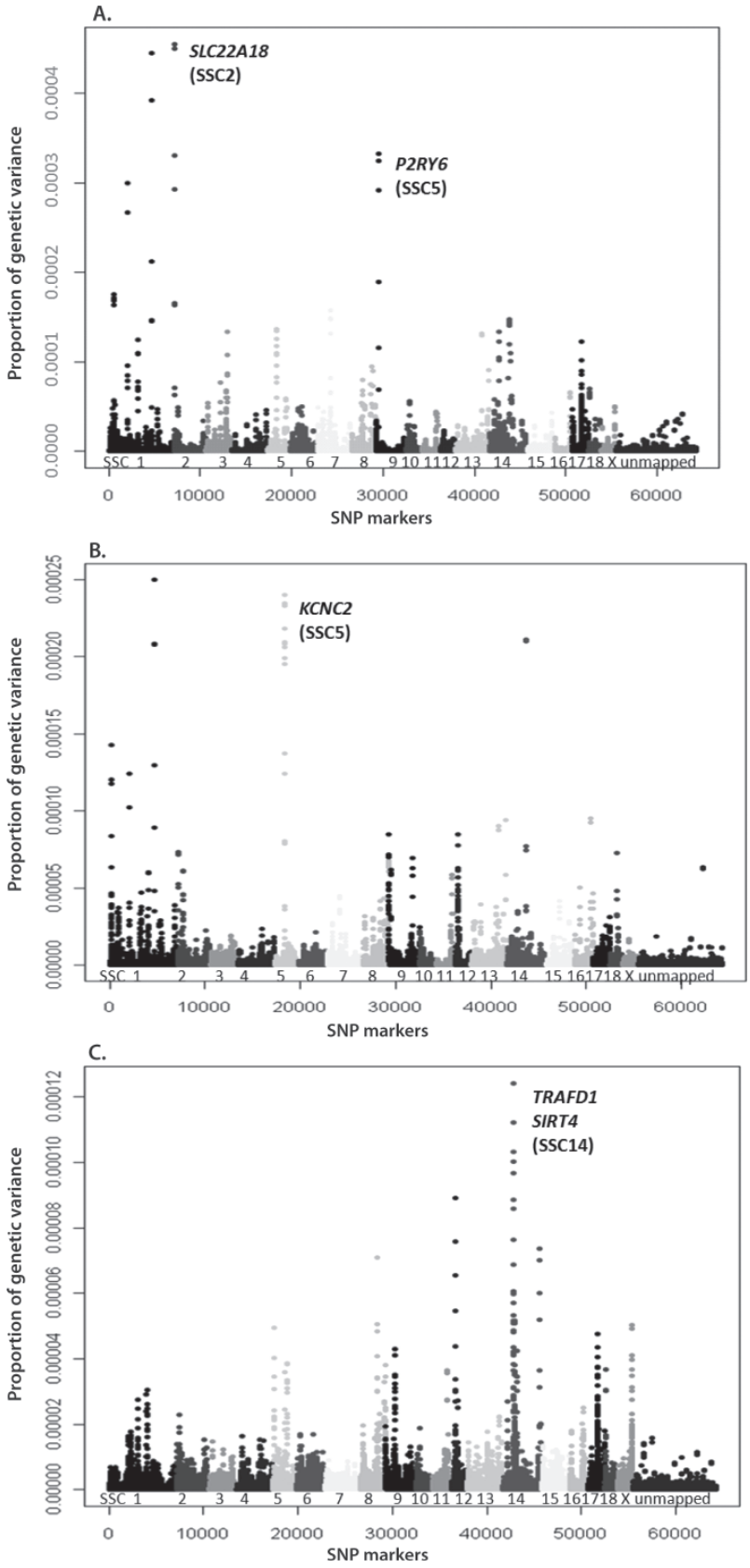

Figure 1. Whole-genome analyses for lifetime reproductive traits recorded to a maximum of 9 parities for maternal pig lines: A) Lifetime total number born; B) removal parity; C) ratio between lifetime nonproductive days and herd life. The x-axis is genomic location of SNP. The y-axis represents the proportion of genetic variance, which is a proportion of the variance in genomic prediction of merit accounted for by using a 5-SNP window. The expected proportion of genetic variance in equally spaced windows along the genome is 8.6×10^{-5}. Different shades represent SNP on different chromosomes from SSC1 (left) to X and on unmapped markers (right). Each spot indicates the proportion of genetic variance contributed by a SNP window of 5 consecutive SNP. *SLC22A18* = solute carrier family 22, member 18; *P2RY6* = P2Y purinoceptor 6; *KCNC2* = potassium voltage-gated channel subfamily C member 2; *TRAFD1* = tumor necrosis factor receptor-associated factor (TRAF)-type zinc finger domain-containing protein 1; *SIRT4* = sirtuin (silent mating type information regulation 2 homolog) 4.

mercial pig maternal lines. Although the animals belonged to 2 genetic lines, population stratification analyses categorized them into 1 population. Moreover, the linkage disequilibrium patterns and haplotype frequencies were identified to be similar between these lines in our earlier large-scale association studies for structural soundness traits (Fan et al., 2009). However, the pedigree information indicated that the lines were separate, so to be careful, they were considered a fixed effect in the model for association analyses in this study.

Many analyses with longevity traits generally consider censoring. However, in the present study, few animals remained in the herd for a longer time than the period of data collection at the farm. Therefore, the censoring was very small in this population. Moreover, all the remaining animals were in parity 9 and above, which is considerably greater than the mean culling parity in US commercial herds today. A small percentage of censored animals do not present a large problem, and similar results for linear models and the survival analysis are expected. Because all the remaining animals attained a very high parity, the majority of their records have already been recorded (Engblom et al., 2010). Theoretically, survival analysis with proportional hazard models is sometimes considered superior for the analysis of longevity traits because it properly accounts for censored observations and nonnormal distributions, and can model time-dependent effects (Serenius and Stalder, 2006). However, hazard models are more difficult to implement than the standard linear models (Guo et al., 2001). Moreover, the survival analysis approaches are not yet available for WGAS. Hence, the present study utilized the recently developed genomic selection methods based on Bayesian approaches for continuously observed phenotypes. Permutation testing using bootstrap samples was used to construct the distribution of the test statistic without requiring strong distributional assumptions. To verify the associations of some SNP at significantly associated QTL regions in this study, we also performed a SNP association analysis using the PROC PHREG method (SAS Inst. Inc., Cary, NC). This validation confirmed significant associations between the traits studied and the SNP located at significantly associated QTL regions that were identified by Bayesian approaches. In this study, the 2 litter size traits, LTNB and LNBA, approached normality because of the large number of categories, whereas removal parity had only 10 categories from parity 0 to 9. These traits could be analyzed by categorical analyses. Nonetheless, the experience with ordered categorical genomic analyses [K. Kizilkaya (Iowa State University, Ames; Adnan Menderes University, Aydin, Turkey), R. Fernando (Iowa State University, Ames), and D. Garrick, unpublished data] is that with more than 3 to 4 categories in a trait, there is little difference in power between analyses based on categorical and continuous distributions. Therefore, the methods used in this study are not expected to have a negative effect on the results and conclusions obtained from this data.

A new genomic selection method, Bayes C (Fernando and Garrick, 2008), was used to analyze the present whole-genome data in this study. This method was derived from the Bayes B approach (Meuwissen et al., 2001), which is sensitive to the given priors of genetic and residual variance. However, Bayes C is much less reliant on priors (Kizilkaya et al., 2010), with much of the information contributing to the posterior coming from the data. Further, a SNP sliding window approach was used to identify the most informative regions because it accounts for linkage disequilibrium between neighboring SNP and has been shown to be better at discriminating important chromosomal effects from spurious effects of a single SNP (Sun et al., 2011). Assuming the experiment had 50% power and that 99% of null hypotheses of no QTL in the SNP window are true, then the probability of false positives from Fernando et al. (2004) is 0.66 for $P < 0.01$ and 0.16 for $P < 0.001$. Accordingly, we expect at least one-half of the reported QTL to be real.

The proportion of phenotypic variance explained by markers was 0.15 for LTNB and LNBA, 0.12 for removal parity, and 0.06 for LNPDR. The lifetime litter size traits (LTNB and LNBA) had moderate (0.15 for both) variance proportions relative to the other traits evaluated in this study. These variance proportions were quite similar to the heritability estimates reported by Nikkilä et al. (2010) on the large population (1,447 animals) from which the studied population (818 animals) was obtained, which suggests that there was sufficient shrinkage from the Bayesian model averaging method to avoid overfitting. In addition, these estimates were comparable with those of other studies in different populations with Landrace (0.19; Johnson and Nugent, 2008) and Large White sows (0.07 to 0.14; Mészáros et al., 2010). Because the sows in the present study were from genetic lines originated by Large White or Large White × Landrace interbreed crossing, the proportion of variation explained by markers did not appear to be overestimated. Further, these estimates were less than the heritability estimates of 0.23 to 0.25 reported in other pig populations (Guo et al., 2001) for lifetime litter size. Therefore, the genetic markers associated with LTNB and LNBA would be considered better markers for selection programs targeting improvements in SPL using these traits than the programs aimed at other SPL measures.

In the present population, many genes at the associated QTL regions (on SSC2 at position 0.2 to 0.8 Mb and on SSC9 at position 6.98 to 7.51 Mb) with lifetime litter size (LTNB and LNBA) are expressed in reproductive tissues and contribute to reproductive processes. For instance, the gene *SLC22A18* in the very highly significantly associated QTL region on SSC2 has been reported to be expressed in the placenta and might lead to reduced fetal embryonic growth in mice (Salas

et al., 2004). A QTL for mummified fetuses was previously located in this region (Holl et al., 2004). The P2Y purinoceptor 6 (***P2RY6***) gene on SSC9 at 6.98 to 7.51 Mb is also believed to have placental functions because of its greater expression in placenta than in the other tissues (GeneAtlasU133A, gcrma No. 208373_S_at). Hence, the genes in these very highly significantly associated QTL regions on SSC2 and SSC9 could be good markers for lifetime litter size in pigs. The *KCNC2* gene containing a QTL region on SSC5 was very highly significantly associated with removal parity. This gene encodes for voltage-gated potassium channel subunits, which are mainly expressed on GABAergic neurons and regulate the release of γ-aminobutyric acid (**GABA**; Goldberg et al., 2005). The neurotransmitter GABA regulates the excitability of GnRH neurons and inhibits the release of GnRH (Zhang et al., 2009). This indicates that the genes regulating the release of GnRH could contribute to some reproductive problems that lead to culling of animals for reproductive reasons. In the present population, 34.1% of culling was due to reproductive problems, such as repeat estrus, late gestation, uterine prolapse, poor mothering, bad udder, discharge of pus from the uterus, difficulty in farrowing, small litter, abortion, and poor weaning performance. The number of lifetime nonproductive days, and hence LNPDR, was considered mainly as days from first service to conception and the weaning to conception interval from each parity. Hence, the problems related to estrus cycle, ovulation, and fertilization could be reasons for nonproductive days. Most interesting of the 10 reproductive genes located at the very highly significantly associated QTL region on SSC14 for LNPDR, *TRAFD1* is one of the negative regulators for the Toll-like receptor signaling pathway. Although the Toll-like receptor signaling pathway is related to innate immunity, several recent studies have shown that this pathway is involved in ovulation, transport of oocytes in the oviduct and during fertilization (Mashima et al., 2005; Herath et al., 2007; Liu et al., 2008; Shimada et al., 2008).

Most of the associated regions in this study were at previously reported QTL regions related to reproductive traits and fat deposition in pig genome build 9. This indicates that the associated genomic regions can regulate fat metabolism, specifically fat deposition. It is well known that optimal fat deposition is required for SPL (Stalder et al., 2005). Leaner pigs cannot recover their body condition after lactation, resulting in further reproductive problems. They cannot tolerate management, environmental, and nutritional deficiencies, and they are more susceptible to physical injuries and further culling from the herd (http://www.hypor.com/en/Breeding/~/media/Files/Hypor/Weaning%20Capacity%20Articles/English/WC11%20Management%20for%20high%20sow%20longevity.ashx). Therefore, the associated QTL regions in this study reinforce the role of fat regulation for lifetime reproductive traits.

The present analyses, using the PorcineSNP60 BeadChip, found that several QTL regions and genes within these regions were associated with some pig lifetime reproductive traits that had a low to moderate heritability. A much greater density SNP chip or a larger population may yield more information about the associated genomic regions for these traits. However, validation studies conducted in other populations are required to confirm that the SNP or genes identified in this study provide information about SPL and can contribute to improved accuracy for genetic evaluation of SPL.

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