Genetic Associations of Farrowing Length in Two Maternal Lines of Pigs

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ABSTRACT: Reproductive success has a direct impact on the economy and profitability of the pork production system. Prolonged farrowing has been negatively correlated with number of piglets born alive and decreased fertility in the subsequent breeding-season. To better understand the genetics involved with birth traits, a genome-wide-association-study (GWAS) was conducted to identify SNPs associated with farrowing length (FL) in two swine breeds. Five chromosomal regions involved with FL were identified using the additive/additive+dominance models in the Landrace breed: on *SSC0/SSC1/SSC2/SSC4/*S*SC13,* and two regions on *SSC15* when the additive+dominance model was tested. For the Large White breed, four regions (*SSC4/SSC9/SSC12/SSCX)* were associated with FL using the additive/additive+dominance models. When we tested the additive+dominance effects, five chromosomal regions were associated with FL: on *SSC*0/*SSC*1/*SSC*4/*SSC5/SSC6*. Identification of markers associated with FL might unravel genes involved with birth traits. Hence, genetic selection can be applied to optimize farrowing time, according to the desirable breeding goals.

Keywords: GWAS; farrowing length; swine; SNP additive and dominance effects

Introduction

During farrowing it is expected that sows will give birth to several piglets in a short period of time. This short period is critical for piglet survivability, as its delay can increase the number of stillborn (Vallet et al. 2010). Several environmental and physiological factors have been associated with farrowing length and consequently with number of piglets born alive (Oliviero et al. 2010). The average farrowing time can range from two to six hours and sows with prolonged farrowing time tend to have poor health status during lactation (van Dijk et al. 2005). Consequently, these sows could have subsequently decreased fertility and increased number of culling due to conception failure, possibly caused by the poor health status (Oliviero et al. 2013). Therefore, problems resulting in a prolonged farrowing duration will have a direct impact in the animal health, affecting, consequently, the economic results.

Endocrine factors are also directly involved with duration of farrowing, as higher levels of circulating oxytocin during parturition are associated with short farrowing time (Castrén et al. 1993). However, stress factors can influence the farrowing time, as epinephrine can interfere with the release of oxytocin, reducing the amount of uterine contraction, increasing the parturition time (Sobestiansky et al. 1998). Duration of farrowing is a low heritable trait (h^2=0.05), with moderated genetic correlation with piglets born alive (r_g= -0.20 $\pm$ 0.01) and high r_g with stillborn (0.66 $\pm$ 0.01) (Holm et al. 2004). Those results indicate the presence of a genetic factor involved with the duration of farrowing. Therefore, genetic selection can be applied to optimize farrowing time, according to the desirable breeding goals.

This study aims to investigate genomic regions associated with farrowing duration in two maternal lines of pigs: Landrace and Large White.

Materials and Methods

Animal Population. Animals used in this study had a complete pedigree composed of 81,534 Landrace (LA) and 48,598 Large White (LW) animals born between 2002 and 2013. Farrowing data used in this study are from two BRF S/A maternal lines, housed in the Santa Catarina State, Brazil. The data comprise parities from 4,840 Landrace and 4,701 Large White, restricted to the first four parity orders, from 3,345 Landrace and 2,953 Large White sows. To estimate additive correction factors, a repeatability animal model was fitted with contemporary group (year, week and order of parity) and the covariate litter size as fixed effects, and the additive, permanent environment and residual as random effects. Contemporary groups with less than five records were removed from the analyses. Repeatability model was fitted using REMLF90 software (Misztal 2003). Additive factors were applied to the phenotypes of 570 Landrace and 432 Large White genotyped animals to obtain corrected phenotypes. For this study, only data from gilts were used.

SNP Genotyping. Genomic DNA was extracted from 2,378 tissue samples using the Invitrogen™ genomic DNA extraction kit, according to the manufacturer's instructions. DNA concentration was measured using the NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA). DNA quality was measured using the 260/280 ratio of 1.8 to 2.0 and 230/280 ratio of 2.0 to 2.3. Samples were diluted to 50 ng/µl for a final concentration of 500 ng and genotyped at GeneSeek (Lincoln, Nebraska, USA) with the Illumina PorcineSNP60 BeadChip (Illumina, San Diego, California, USA). The PorcineSNP60 V1 and V2 assay contained

61,177 common SNPs with mean spacing of 43.4 kb and median spacing of 28 kb between SNPs.

Animal and SNP Quality Control. A quality control (QC) filtering was applied to animals and SNPs within each breed. Genotype quality assurance was performed using the open source R statistical environment (R Core Team 2012) and PLINK (Purcell et al. 2007). Samples with >10% missing genotype, with heterozygosity outside a 95% confidence interval, IBD >0.9 and paternity inconsistence were removed from the analysis. SNPs were removed if they fail in >2% of the animals, minor allele frequency <3% or failing Hardy-Weinberg Equilibrium ($p<10^{-6}$).

A Multi-Dimensional Scaling plot (MDS) was constructed to verify population substructure and genetic variation among animals within breed prior to the analysis (Figure 1). Samples from 46 Landrace and 70 Large White animals were removed due to low genotype call rate, leaving 1,168 LA (91 males, 1,077 females) and 1,094 LW samples (114 males, 980 females) for the analysis. For the common set of 61,177 SNPs shared among the Illumina PorcineSNP60K Version 1 and 2, the QC was applied and removed 20,135 SNPs from LA and 24,725 SNPs from LW, remaining, respectively, 41,042 and 36,452 SNPs for the association studies.

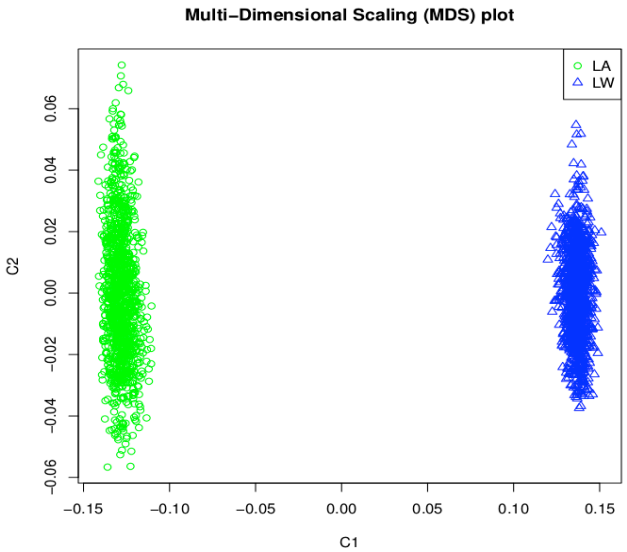

Figure 1: Multi-Dimensional-Scaling (MDS) plot of the Landrace (LA) and Large White (LW) animals, constructed using only markers with low LD ($r^2<0.2$). Population structure and diversity among and between breeds are shown.

Statistical Analysis. A general mixed model accounting for additive and dominance effects of the SNPs was performed using QxPak 5.05 (Pérez-Enciso and Misztal 2011) to test for association of each SNP with farrowing length in both Landrace and Large White populations. Significance was considered if $p<5x10^{-5}$ (WTCCC 2007).

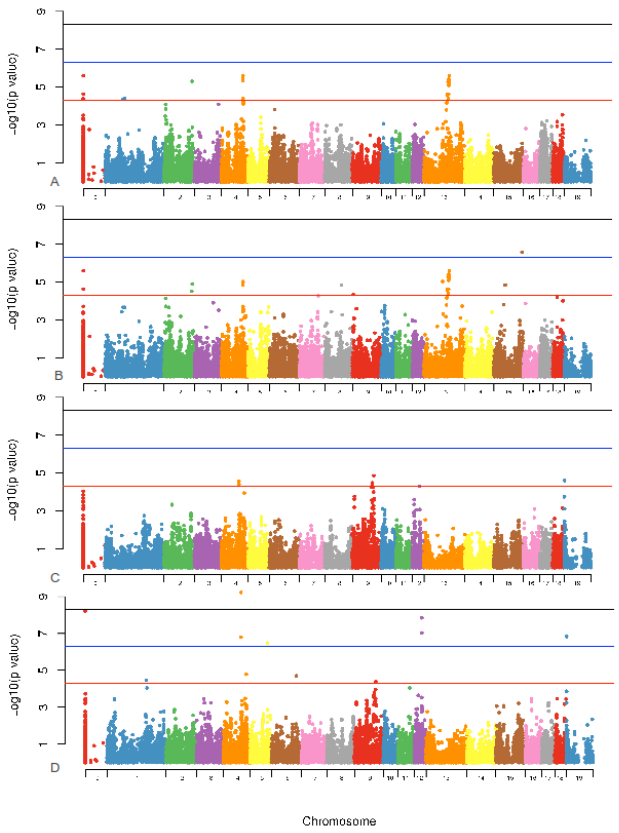

Figure 2: A) GWAS fitting the SNP additive effect for the Landrace breed, B) GWAS fitting the SNP additive and dominance effects for the Landrace breed, C) GWAS fitting the SNP additive effect for the Large White breed, D) GWAS fitting the SNP additive and dominance effects for the Large White breed.

Results and Discussion

The average farrowing time in the Landrace and Large White breeds was 5.57±2.30 and 5.45±1.9 hours, respectively. Results from the association test in the Landrace breed identified four chromosomal regions associated with farrowing length with the additive model, in addition to tree SNPs with unknown location (included in the *SSC0*) (Figure 2A). Two markers located on *SSC*1 at 97,143,269 bp and 104,709,048 bp were in high LD (D'=1), being considered a single region. In this region, the gene *HTR1B* was found to be related to serotonin, a hormone involved in parturition (Marian et al. 2008). The second region was composed by a SNP on *SSC2* located at 150,370,400 bp ($p<4x10^{-6}$). This marker is located on the *FGF1-201* predicted gene. The third region was on *SSC*4 composed of 8 SNPs spanning 739 kb, from 114,607,395 to 115,346,647 bp ($p<2x10^{-5}$), with the highest peak from 114,755,650 to 114,841,135 bp ($p<2x10^{-6}$). The LD levels between the 8 markers were ~D' >0.97. Four genes were located in this region (*TSH-Beta, NRAS, AMPD1* and *BCAS2*), however, none of them have a direct involvement with farrowing length. Nevertheless, during parturition, the levels of the thyroid hormone regulated by the *TSH-Beta* were found in lower concentrations in comparison with the basal levels, indicating its possible role during birth

(Messer et al. 1998). The fourth region was from 121,983,388 to 134,291,323 bp on *SSC*13 (D' >0.91) composed of 28 SNPs ($p<3x10^{-5}$). This region is in a moderate LD (D' >0.56) with an intronic SNP (*rs81330464*) on the Oxytocin Receptor (*OXTR),* suggesting its possible role with duration of birth. The *OXTR* plays an important role in the uterine contraction and parturition length, being an important candidate gene associated with farrowing (Bakermans-Kranenburg et al. 2008). *TSH-Beta* and *OXTR* are part of the same Retroactive Ligand-Receptor Interaction pathway, which also involves other hormones related with parturition.

When the dominance effect was also included in the model, all the regions associated with the additive effect were still significant except for SSC1, and five additional regions in four chromosomes became significant (Figure 2B): one on *SSC*2 at 148,611,135 bp ($p<3x10^{-5}$), one on *SSC*8 at 89,453,323 bp ($p<1x10^{-5}$), one on *SSC*9 at 4,653,168 bp ($p<4x10^{-5}$) and two regions on *SSC15*, between 58,085,034 to 60,473,765 bp ($p<1x10^{-5}$) and at 148,962,520 bp ($p<2x10^{-7}$). The *MAK16* gene was located into the first associated region of *SSC15,* however, no direct function of this gene has been identified with farrowing length.

In the Large White breed, fitting the additive effect of the SNPs, four regions were associated with farrowing length (Figure 2C): one was located on *SSC4* between 93,397,427 to 93,498,852 bp ($p<3x10^{-5}$), with high levels of LD (D'= 1) among the markers. The second region was located on *SSC*9 between 105,553,490 to 113,942,105 bp ($p<4x10^{-5}$), with high LD among markers (D'= 0.9). The third region was on *SSC*12 at 40,135,149 bp ($p<4x10^{-5}$). The fourth one was on the beginning of *SSC*X ($p<2x10^{-5}$). No genes were found in those regions that could explain the variation on farrowing time.

When dominance was also fitted in the model, eight chromosomal regions were identified as associated with farrowing length (Figure 2D): on *SSC0, SSC1, SSC4, SSC5, SSC6, SSC9, SSC12* and *SSCX*. The regions on *SSC*4 and *SSC*12 were also associated with the additive model. However, when fitting the additive + dominance model, the region on *SSC*4 (93,397,427-93,498,852 bp) presented an important dominance effect ($p<5x10^{-10}$). Another region on *SSC*4 located between 121,586,372 – 121,607,174 bp ($p<1x10^{-5}$) was also associated using the a+d model. The markers located on *SSC*5 (91,782,152 bp - $p<3x10^{-7}$), *SSC*6 (133,929,215 bp – $p<3x10^{-5}$) and a SNP on the sexual chromosome at 2,095,311 ($p<1x10^{-7}$) were also associated with farrowing time. In addition to that, a region on *SSC*12 spanning 37Kb between 40,020,679 to 40,057,790 bp ($p<9x10^{-8}$) were also identified to be involved with farrowing time in the Large White population. No genes have been annotated in the regions associated with farrowing in the Large White animals.

None of the regions associated with farrowing length in the Landrace breed were also associated in the Large White breed.

Conclusion

Up to this moment, no genomic regions have been previously studied or described as associated with parturition length in multiparous livestock species. Our results indicate possible involvement of breed specific genetic regions associated with this phenotype in swine. The *TSH-Beta* and the *HTR1B* were identified as suggestive positional and functional candidate genes involved with farrowing length in Landrace animals. Even knowing that several chromosomal regions were associated with farrowing length in the Large White breed, no annotated genes were located in the surrounding areas. Further studies are being conducted to validate those results in an independent population, in additional to other swine breeds.

Acknowledgements

This work was supported by the project 02.09.70.600.01 from the Brazilian Agricultural Research Corporation (EMBRAPA). The authors are grateful to A. L. Tessmann for technical assistance and to the Embrapa Bioinformatics Multi-Users Laboratory (LMB) for the infra-structure and assistance in the implementation of computational solutions. R. Zanella received a BJT scholarship from the *Science without Borders* Program - National Council for Scientific and Technological Development (CNPq, grant nº 373167/2012-1).

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