Whole-Genome Association Analyses For Sow Lifetime Production, Reproduction and Structural Soundness Traits Using the PorcineSNP60 BeadChip

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Introduction

Increasing sow productive life or longevity is one way to improve the production efficiency for commercial sow production units. In the USA, the culling rate increased from 44.6% in 2000 to 51.0% in 2007 (www.thepigsite.com) due primarily to increases in reproductive and leg structure problems. Lifetime prolificacy and structure traits are correlated to sow longevity. Heritability estimates for these traits were low to moderate (0.1-0.35) using multiple trait animal models (Nikila *et al.* 2010). QTL mapping (http://www.animalgenome.org/cgi-bin/QTLdb) and candidate gene approaches (Rothschild *et al.* (1996); Fan *et al.* (2009)) were conducted to explore the complex genetic architecture of lifetime production, reproduction and structural traits in pigs. Though some progress has been obtained in earlier studies, the development of SNP chips (e.g., PorcineSNP60 BeadChip) and the extensive use of advanced statistical tools using Bayesian methods may provide better ways to dissect the genetic structure for complex traits like sow productive lifetime. The objectives of the present study were to identify genetic markers and/or chromosomal regions on a whole genome level associated with sow lifetime production, reproduction and leg structure soundness traits using the 60K PorcineSNP60 BeadChip.

Material and methods

A total of 820 commercial female pigs (408 Large White, 412 Large White × Landrace) from a single farm unit of Newsham Choice Genetics (West Des Moines, IA, USA) were genotyped by GeneSeek Inc (Lincoln, NE, USA) using the porcine 60K SNP chip (Illumina, San Diego, CA, USA). The traits included first parity total number born (TNB1), lifetime total number born (LTTNB) and lifetime number born alive (LTNBA), to a maximum of nine parities, recorded on animals from 2005 to 2009. The overall leg action, and front and rear leg pasterns were scored on nine point scales (Fan *et al.* (2009)) by two trained scorers when animals reached ~125kg body weight. A set of quality control measures (call rate ≥ 80%, Gentrain score > 0.4) were implemented on the 64,232 SNPs and a total of 57,814 SNPs qualified for association analyses. The analyses were implemented with a Bayesian approach for 50,000 (for reproduction traits) and 200,000 (for leg structure traits) Markov chains that fitted mixture models whereby loci were assumed to have no effect with probability 0.995 or simultaneously influenced the trait with probability 0.005 ("Bayes C option" in GenSel, http://bigs.ansci.iastate.edu). Fixed effects of genetic line and season were used for reproduction traits, whereas genetic line, scoring date, scorer and body weight as a covariate were used for leg structure traits. A "Predict" option in GenSel was used to

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estimate the proportion of genetic variance accounted for by each window of five consecutive SNPs within this population. The genetic variance proportion for an individual SNP was computed by dividing its estimated genetic variance by the total additive genetic variance of that particular trait. The estimated proportion of genetic variance contributed by each SNP or by windows of five consecutive SNPs were plotted against genomic locations of the markers using R software (http://www.r-project.org). The genomic locations with highest genetic variance were considered for further investigation for each trait. The gene search and their functional annotations were carried out in these chromosomal regions using the *Sus scrofa* 9 genome build and DAVID online (http://david.abcc.ncifcrf.gov), respectively.

Results and discussion

The summary of association results for lifetime production, reproduction and leg structure traits by single SNP and five consecutive SNP windows are presented in Tables 1 and 2, respectively. Proportion of genetic variance by SNP markers for LTTNB is in Figure 1. The proportion of phenotypic variance explained by 250-300 markers was moderate for LTTNB, LTNBA, leg action and pasterns, but it was low for TNB1 (Table 1). Genetic markers for lifetime litter size and structural traits could be considered as important markers for longevity selection programs. Individual SNP marker analyses found that the SNP MARC0067803 associated with LTTNB and LTNBA was within the Annexin 6 (*ANXA6*) gene on SSC16. *ANXA6* is involved in feto-maternal ion transport especially of placental chloride conductance (Riquelme *et al.* (2004))**.** The genes *MEF2C*, *PAX5* associated with TNB1 are involved in embryonic development and age at puberty (Kuehn *et al.* (2009)), respectively. A family of *HOX* genes (*HOXA*1-13) on SSC18 in the vicinity of SNP MARC0033103 were found to be associated with leg action. Hox genes exert essential roles on the morphogenesis of skeletal structure along the antero-posterior axis (Favier & Dolle, (1997)). Similarly, genes involved in bone formation (*BMP2*) and locomotory behavior (*BSX9*) (Sakkou *et al.* (2007)) were associated with pasterns in this study.

SNP window analyses with five consecutive SNPs found several chromosomal locations for further analyses to identify candidate genes. A total of 33, 26, 16, 61, 63 and 41 genes were found in the associated candidate chromosomal regions mentioned in Table 2 for LTTNB, LTNBA, TNB1, overall leg action, and front and rear pasterns, respectively. The SNP window has the advantage that it accounts for linkage disequilibrium between neighbouring SNPs and may therefore be better at discriminating the chromosomal effects from spurious effects. The SNPs on SSC2 were less associated with LTTNB than the SNP on SSC16 in the plot of single SNP effect (Figure 1, left panel). However, the region on SSC2 showed relatively higher association with LTTNB than the region on SSC16 by the SNP window approach (Figure 1, right panel). This indicates the SNPs in the associated region on SSC2 for LTTNB might be in higher LD than the SNPs on SSC16. Though few coding genes were annotated to be related to reproduction and leg structure traits (Table 1 and 2), many genes in the identified chromosomal regions might have unknown novel functions for these complex traits.

Table 1: Whole genome individual SNP marker analyses in a study involving commercial maternal pig lines

Traits	Proportion of phenotypic variance explained by 250-300 markers	SSCs for top 10 SNPs in descending order	Interesting genes within <0.4MB of top SNPs (SSC)*
LTTNB	0.18	16, 14, 13, u**, 13, 8, 1, 11, 18, 1	*ANXA6* (16), *FUT9* (1), *ZIC3* (11), *ZIC5* (11)
LTNBA	0.18	16, 13, 14, 8, 5, 11, 18, 13, u, 14,	*ANXA6* (16), *ZIC3* (11), *ZIC5* (11)
TNB1	0.003	u, u, 2, 16, 2, 2, u, 1, 1, 4	*MEF2C* (2), *PAX5* (1), *MELK* (1)
Leg action	0.29	16, 16, 2, 6, 9, 18, 13, 13, 18, 16	*FHL3* (6), *HOXA1-13* (18)
Front pasterns	0.33	17, 17, 15, 12, u, u, 12, 12, 16, 16	*BMP2* (17)
Rear pasterns	0.12	17, 9, 10, 17, 1, 15, 15, 15, u, u	*BMP2* (17), *BSX* (9)

*SSC: Pig chromosome; *ANXA6*: Annexin 6; *BMP2*: Bone morphogenic protein 2; *BSX*: Brain specific homeobox; *FUT9*: Alpha-(1,3)-fucosyl transferase; *FHL3*: Four and a half LIM domains protein 3; *HOXA*: Homeobox A**;** *MELK*: Maternal embryonic leucine zipper kinase; *MEF2C*: Myocyte enhancer factor 2C; *PAX5*: Paired box protein 5; *ZIC*: Zinc finger. **u: SNP are unmapped to any chromosome.

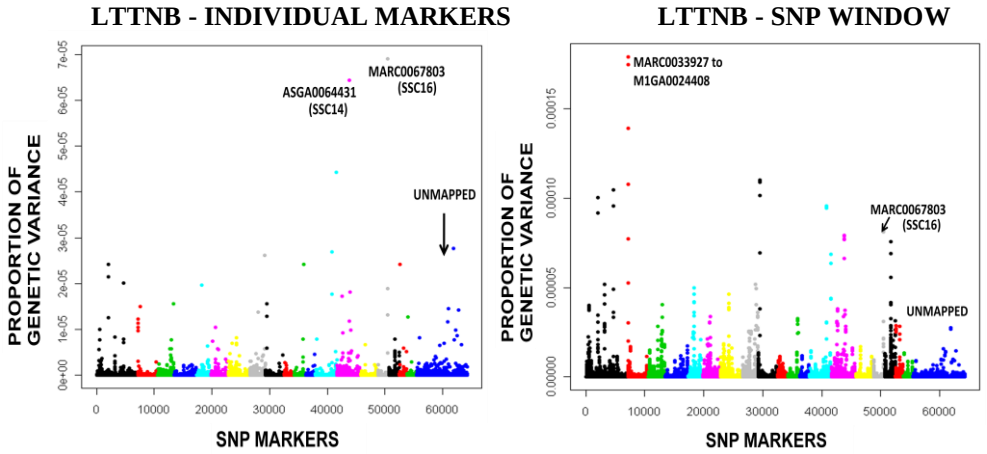

Figure 1: An example of plots for individual SNP (left) and windows of five consecutive SNPs (right) analysed from commercial maternal pig lines.

(Note: The X-axis is genomic location of the SNPs, and the Y-axis represents proportion of genetic variance. Different colours represent SNPs on different chromosomes from SSC 1 to X and unmapped markers. Y- axis scale is different on the two figures.)

Table 2: Whole genome consecutive five SNP window analyses in a study involving commercial maternal pig lines.

Traits	No. of associated candidate regions	SSCs	Some genes within the candidate regions (SSC)*
LTTNB	11	1, 2, 8, 9, 13, 14, 16, 17	*NADSYN1* (2), *ANXA6* (16)
LTNBA	10	1, 2, 5, 9, 13, 14, 16, 17	*NADSYN1* (2), *ANXA6* (16)
TNB1	5	2, 3, 7, 13	*MEF2C* (2)
Leg action	15	2, 3, 5, 6, 9, 13, 14, 15,16, 18	*HOXA1-3* (18), *FHL3* (6)
Front pasterns	24	1, 5, 8, 9, 12, 14, 16	*BMP2* (17), *SOX9* (12)
Rear pasterns	11	1, 3, 4, 6, 9, 10, 15, 17	*BMP2* (17), *BSX* (9)

**ANXA6*: Annexin 6; *BMP2*: Bone morphogenic protein 2; *BSX*: Brain specific homeobox; *FHL3*: Four and a half LIM domains protein 3; *HOXA*: Homeobox A; *MEF2C*: Myocyte enhancer factor 2C; *NADSY1*: Glutamine-dependent NAD(+) synthetase; *SOX9*: SRY (sex determining region Y)-box 9.

Conclusions

The present analyses provided chromosomal regions and candidate genes (e.g., *MEF2C* and *BMP2)* associated with sow lifetime production, reproduction and structural soundness traits. This list of genes could be considered for future genetic marker association studies for the complex traits involved in this study using other populations with larger size or different genetic backgrounds and in different farm management conditions. Such validation studies are needed first before use of these genes in marker assisted selection programs.

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