

MAPPING OF QTLs FOR PROLIFICACY TRAITS ON SSC8 USING A CANDIDATE GENE APPROACH

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INTRODUCTION

Sow prolificacy is a critical factor affecting the profitability of the swine industry. While there have been large increases in productivity attributable to improvement in genetics and management, litter size has remained unchanged for several decades. Genetic improvement has been difficult to achieve using traditional selection methods due to the low heritabilities of traits involved (Roehle and Kennedy, 1995). Meishan pigs are perhaps one of the most prolific breeds of pig in the world. They reach puberty at 2.5 - 3 months of age, achieve high embryo survival rates, and a large litter size of 15 - 16 pigs. Although a few Meishan-synthetic gilts appeared on the market in the early 1990s, the promise of a commercial boost to litter productivity has not yet been realized. The reason is that the large advantages in litter size are finely balanced with equally large disadvantages in growth and carcass value. Therefore, the identification and introduction of only alleles for the high prolificacy of Taihu pigs into highly productive European/American breeds would have obvious commercial value.

Using a whole genome scanning approach with microsatellite markers, it was determined that there are three quantitative trait loci influencing ovulation rate on porcine chromosome 8 (Rathje *et al.*, 1997 ; Rohrer *et al.*, 1999 and Wilkie *et al.*, 1999). These indicate the importance of screening porcine chromosome 8 for additional markers, especially candidate genes for prolificacy in pigs. The gene encoding the gonadotropin-releasing hormone receptor (*GNRHR*) has been mapped to chromosome 8. Jiang and colleagues (2001) obtained an almost complete sequence (3993 bp, excluding intron 1) of the porcine *GNRHR* gene using PCR-based comparative genomic walking and inverse genomic walking approaches. Twelve genetic polymorphisms were detected by sequencing of pooled DNA of Chinese Taihu and Large White pigs. An F2 population of Meishan x Large White pigs was genotyped for a TG deletion/insertion in the promoter region, and a C/G substitution in the 3'UTR. A significant association of the C/G substitution with number of corpora lutea at first parity was observed.

Measuring conservation of contiguous sets of autosomal markers between human and porcine genomes has revealed that SSC8 is homologous to a region from approximately 0 - 170 Mb on human chromosome 4 (HSA4) (Jiang *et al.*, 2002 submitted). Based on human genome mapping information, several additional functional genes in this region (*AREG*, *FGG* and *STE*) were found to be involved in reproduction. Amphiregulin (*AREG*), a member of the epidermal growth factor (*EGF*) family, is one of the genes important for appropriate embryonic attachment (Giudice, 1999). Granulosa cells from ovarian follicles were shown to express and

secrete fibrinogen gamma chain (FGG) under the control of FSH (Parrott *et al.*, 1993). Purinton and Wood (2000) reported that ovine fetal hypothalamus and brainstem contain estrogen sulfotransferase (STE) in brain regions important for hypothalamus-pituitary-adrenal axis control. The targeted disruption of murine estrogen sulfotransferase caused structural and functional lesions in the male reproductive system (Qian *et al.*, 2001). *AREG*, *FGG* and *STE* have been mapped to human chromosome 4q13 - q21, 4q28 and 4q13.1, respectively. Here we report the detection of nucleotide polymorphisms in porcine *AREG*, *FGG* and *STE* genes, and associations of these polymorphisms with prolificacy in an F2 population of Meishan x Large White pigs.

MATERIALS AND METHODS

Primer design and genetic polymorphisms. Primer sequences for amplifying the PCR products in porcine *AREG*, *FGG* and *STE* genes are as follows : *AREG*, F-CCAAAAGAAAAGAAAAGGGAGGCA and R-GCGGCTTTTCCCCACATCGTTCACC ; *FGG*, F-GTTTGTAGCATGTTAAAAATTCGC and R-ATTTCCAGACCCATCAATTTCA; and *STE*, F-CCCAGCCTCAGCAATAGTATTAATA and R-ACTTATGTCTTCGTATCTAG CCGC. Genomic DNA (~50 ng) was amplified in a final volume of 10 µl containing 3 pmol of each primer, 200 nM dNTPs, 2.5 mM MgCl₂, 50 mM KCl, 10 mM Tris HCl, 0.1 % Triton X-100 and 0.5 U of *Taq* polymerase. After denaturation at 94°C for 3 min, 30 amplification cycles were performed comprising denaturation at 94°C for 30 sec, annealing at 57°C for 30 sec and extension at 72°C for 30 sec, followed by a further 5-min extension at 72°C. Direct sequencing of PCR products from 2 pooled DNA samples of 16 Chinese Erhualian and 16 European Large White pigs was performed using an ABI 377 automatic sequencer (Applied Biosystems, Foster City, USA) and standard protocols.

Genotyping. PCR-RFLP, Bi-PASA and PCR-DSCP techniques were used to genotype markers in the porcine *AREG*, *FGG* and *STE* genes, respectively. In the PCR-RFLP assays, 5 µl PCR products were digested with 5 U *StyI* for the G/A substitution at the porcine *AREG* gene. Inner primers for the Bi-PASA genotyping of a TCT indel in *FGG* were: GGGCGGGGG CAATCTGAAAAGAAAGAA and GGGCGGGGGCGTAAAATCATCTTCTTT. Both PCR-RFLP and Bi-PASA products were then examined by electrophoresis on 1.5 % agarose gel with 1X TBE buffer, while PCR products of the *STE* gene were examined using 8 % acrylamide gels. The gels were stained with ethidium bromide and photographed.

Marker-trait association analysis. Estimates of the effect of polymorphisms on sow reproduction traits were obtained by fitting a linear model including genotypes at the three loci described above, and year as fixed effects and, for all traits except age at first mating, linear covariates for age at first mating, weight at laparoscopy or age at farrowing, using the GLM procedure of SAS. Traits analyzed were entire teats (ET), age at first mating (AFM), number of mating (NOM), number of corpora lutea observed by laparoscopy (NCL), gestation length (GL), number born (NB) and number born alive (NBA). All traits except AFM were recorded at 1st and 2nd parity, with records at the two parities being considered as separate traits, denoted NCL1, NCL2, GL1, GL2, etc.

RESULTS AND DISCUSSION

DNA direct sequencing of two pooled samples from Meishan and Large White pigs revealed a C/T substitution in *AREG*, a TCT indel in *FGG* and a C/A substitution in the *STE* gene. The C/T substitution in the *AREG* gene can be detected with the restriction enzyme *StyI* (figure 1A). The PCR products of the *AREG* gene have also a common cut site for *StyI* for all animals, which serves as an internal control. In *FGG* genotyping, all animals show a common band amplified with the outer primers, while allele specific bands were generated with allele specific primers (figure 1B). PCR-DSCP analysis revealed three genotypes in the *STE* gene : lane 1, heterozygous ; lane 2, homozygous for allele1 and lanes 3 - 6, homozygous for allele 2 (figure 1C). All polymorphic markers were shown to be under simple genetic control by segregation analysis in multiple three-generation families of Meishan -Large White crosses.

Data analysis revealed that an allele dominant in Meishan pigs at the *FGG* gene (allele A1) was associated with a desirable effect on number born and number born alive in the second parity. All estimates summarized in table 1 are relative to the homozygote A2A2 which was set to zero for estimability. The homozygote (A1A1) and heterozygote (A1A2) genotypes were associated with 2.71 and 3.27 piglets ($P = 0.0469$) more in number born and 2.29 and 3.20 ($P = 0.0266$) more in number born alive than the homozygotes with the undesirable allele (A2). However, a Meishan allele (A1) at the *AREG* gene was associated with an undesirable effect on number born in the first parity : 1.65 (A1A1) and 2.06 (A1A2) piglets fewer born than for A2A2 homozygotes (table 1). The *STE* gene showed overdominance effects on both GL1 and ET ($P < 0.0002$ and $P < 0.0603$).

All of the nucleotide polymorphisms analyzed in the porcine *AREG*, *FGG* and *STE* genes were located in introns. Although the SNPs in introns do not directly alter any functionally important amino acid residue, they may prove useful as markers for functional cSNP via linkage disequilibrium mapping. In addition, some introns play a role in regulating gene expression and thus their constituent SNPs may be directly related to functional variation. More generally, the present study provides evidence to support the existence of QTLs for prolificacy traits on porcine chromosome 8. All of the results indicate the importance of porcine chromosome 8 for additional mapping.

Table 1. Significant associations between candidate genes and reproductive traits in the Roslin Meishan x Large White population

Gene	Trait	A ₁ A ₁	A ₁ A ₂	LS Means	Type III SS Pr > F
AREG	NB1	-1.65 *	-2.06 **	12.95	0.0534
	NOM1	-0.20 *	-0.18 *	1.34	0.0298
FGG	NB2	2.71 **	3.27 **	11.13	0.0469
	NBA2	2.29 *	3.20 ***	10.43	0.0266
STE	GL1	0.28 (ns)	2.96 ***	118.1	0.0002
	ET	-0.06 (ns)	0.46 **	14.77	0.0603

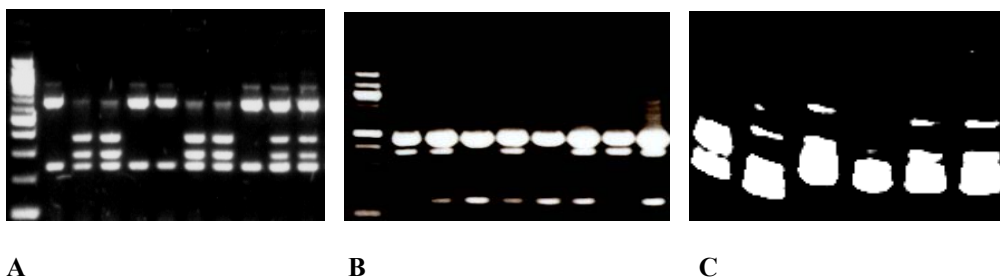


Figure 1. Genotyping of nucleotide polymorphisms in the porcine *AREG*, *FGG* and *STE* genes using PCR-RFLP (A), Bi-PASA (B) and PCR-DSCP (C) techniques (see text for details)

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