A MEISHAN POSITIVE QTL FOR PROLIFICACY TRAITS FOUND AT THE *NCOA*1 LOCUS ON SSC 3.

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INTRODUCTION

Sow productivity, specifically litter size, is of great importance to the success of the swine industry. Sow productivity, defined as the number of live births per sow per year, involves the animal's age at puberty, conception rate, ovulation rate, embryo survival, number of piglets born, number stillborn, number born alive, birth weight, and weaning to service interval. The Chinese Meishan pig has a lower carcass value by Western standards but has a litter size approximately 4 piglets larger than North American breeds, 0.4 fewer stillborn, matures early, and commonly has two litters per year, all economically important traits. The incorporation of Meishan characteristics into North American breeding lines has been only moderately effective; common cross breeding programs have failed to incorporate these favourable traits while avoiding the deleterious carcass quality traits inherent in the Meishan animals.

Reproductive traits are known to be controlled by many genes. A number of genes have been investigated as possible sources of genetic improvement for prolificacy traits. The positive association found between the estrogen receptor gene (*ESR*) and litter size in pigs (Rothschild *et al*., 1996) is a significant finding with regard to potential increases in animal productivity. The porcine gonadotrophin-releasing hormone receptor gene (*GNRHR*) has also been found to be associated with the number of corpora lutea formed (Jiang *et al*., 2001). There are genes that map to SSC 3 that have been identified as being associated with reproductive traits, such as the follicle stimulating hormone receptor gene (*FSHR*) (Remy *et al*., 1995) and the luteinizing hormone/choriogonadotropin receptor gene (*LH-CGR*) (Yerle *et al*., 1992). LH-CGR is involved in signaling pathways in ovarian follicles (Rajagopalan-Gupta *et al.*, 1998). A project involving porcine genome scanning for quantitative trait loci (QTL) associated with reproduction in swine, reported in Rohrer *et al*. (1999), identified 5 regions of which 4 showed Large White composite QTL alleles to be superior to Meishan QTL alleles for ovulation rate.

The nuclear receptor coactivator 1 gene (*NCOA*1), also know as the steroid receptor coactivator 1 gene (*SRC*1), is a candidate gene for influence on swine reproduction traits and has been mapped to SSC3. The human *NCOA*1 gene is comprised of 22 exons with a total genomic length of 183.4 Kb, and a cDNA length of approximately 4721 bp. The nuclear receptor coactivator-1 gene is a likely choice to be investigated as a candidate gene for influence on swine prolificacy traits. The NCOA1 complex interacts with the DNA-bound estrogen receptor serving to enhance its transcriptional activation function (Leo and Chen, 2000). The NCOA1

protein performs histone acetylation and interacts with another acetyltransferase, p300/CBP, in the process of exposing the otherwise inaccessible chromatin (Spencer *et al.*, 1997). Thus, the NCOA 1 protein enhances activity of the ESR receptor that, in turn, stimulates the transcription of specific estrogen-responsive genes and mediates subsequent physiological responses (Nephew *et al.*, 2000). Here we report on a Meishan positive locus associated with prolificacy traits found on SSC 3 at the *NCOA*1 locus.

MATERIALS AND METHODS

Primer Design. Through GenBank, a DNA sequence database provided by the National Centre for Biotechnology Information (NCBI), we have been able to obtain the entire genomic and cDNA sequence of the human *NCOA*1 gene. Using DNA sequence comparison tools available from NCBI we identified cattle sequences that have high homology to the human *NCOA*1 sequence and designed universal oligonucleotide primers that are able to amplify corresponding fragments of the porcine genome. Primer design was conducted using the online oligonucleotide design tool Primer3 (Rozen and Skaletsky, 1998). The forward primer used has the sequence 5' AGGGGCTACCCTCCTGTAAG, and the reverse primer is 5' CTTCTCTGCCAGTTCTCCAGTC.

Polymerase Chain Reactions. A fragment of exon 11 of the *NCOA*1 gene was amplified using the specific primer sequences cited previously. The PCR conditions consisted of 10 µL reactions each consisting of approximately 60 ng genomic DNA, 75 ng of both the forward and reverse primers, 200 nM dNTPs, 2.5 mM $MgCl_2$, 1.0 µL 10X PCR buffer, and 0.5 U AmpliTaq DNA Polymerase (Applied Biosystems, Foster City, USA). After an initial denaturing step of 94°C for 3 minutes, the elongation cycling conditions for the PCR reaction were 8 cycles of touchdown at 94 °C for 30 s, 63 °C-56 °C for 30 s, 72 °C for 30 s, and 32 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. An elongation step of 72 °C for 5 minutes was included for the final step.

Sequencing of Amplified DNA Fragments. The designed primers were used to amplify and sequence DNA fragments from pooled porcine genomic DNA. The two DNA pools consisted of either 20 Ehuralien or 20 Landrace samples. For each mix the DNA was prepared from blood samples. Once DNA fragments had been cleanly amplified, the fragments were sequenced using an ABI 377 automated sequencer located at the Guelph Molecular Supercentre, University of Guelph. The sequences obtained from the Ehuralien and Landrace pooled DNA samples were compared for any polymorphisms and intron/exon borders were determined.

Genotyping of Animals. Within exon 11 a single nucleotide polymorphism (a T/C substitution) was identified. The PCR-RFLP technique was determined to be the most effective method of genotyping animals for the polymorphism. The restriction enzyme *Rsa*1 was identified to be able to cleave the DNA if a cytosine base is present in place of thymine, as shown in Figure 1. Restriction digestions were carried out at 37 °C for 3 hours.

Animal Population. The animal population was developed by the Roslin Institute, Scotland, and consisted of a Meishan x Large White three generation crossbreeding population with 30

grandparent animals of which 16 were pure Meishan and 14 were pure Large White. The 30 grandparent pigs and 220 F_2 sows were used for genotyping and the results analyzed for association with phenotypic data regarding prolificacy traits previously recorded. Statistical analysis was completed using the genotyping data from 178 animals from the Roslin population for which ovulation rate data was recorded.

RESULTS AND DISCUSSION

Sequencing of PCR-amplified DNA from the Meishan and Large White pooled samples revealed a T/C single nucleotide polymorphism (SNP) located in exon 11 of the *NCOA*1 gene. The SNP was determined to be a silent mutation, and analysis was continued based on the logic of using the mutation as a molecular marker.

Statistical analysis was done on the *NCOA*1 genotyping records of 178 animals from the Roslin Meishan x Large White population**.** Analysis was done using Statistical Analysis Software (SAS), with a model fitting the effects of weight at laparoscopy and year with the *NCOA*1 genotype on ovulation rate. The A1A1 genotype (T/T) was found to account for an increase of 2.17 corpora lutea (CP), significantly different from the A2A2 genotype (C/C) value of 16.45 CP (p=0.0087), while the A1A2 genotype (T/C) showed an average increase of 0.90, CP although this increase was not found to be significant. Analysis of the population genotypes and the recorded number born (NB) showed a similar trend, but the results were not found to be significant. The A1A1 genotype showed an increase of 1.82 NB, approaching significance (p=0.1045) as compared to the A2A2 genotype value of 12.38 NB. The A1A2 genotype had an increase of 0.90 NB.

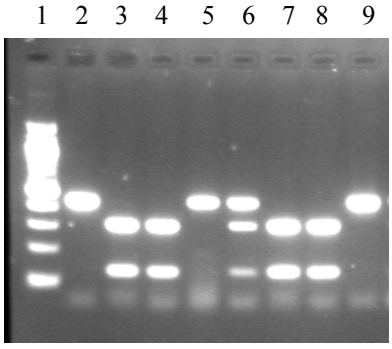

Figure 1. An agarose gel showing the *NCOA*1 exon 11 *Rsa*1 polymorphism in eight grandparent animals. Lane 1 contains a 100 bp standard marker, lanes 2, 5, and 9 refer to Meishan (M) animals with the genotype A1A1, lane 6 refers to a M animal with the A1A2 genotype, and lanes 3, 4, 7, and 8 refer to Large White animals with the genotype A2A2

A nuclear receptor coactivator enhances transcriptional activation by interacting with nuclear receptors bound to DNA. The nuclear receptors that are affected, such as the estrogen receptor, androgen receptor, and thyroid receptor, play important roles in a wide variety of biological

processes including development, homeostasis, and reproduction (Leo and Chen, 2000). The negatively charged DNA has a strong interaction with the positive charge of the histone N termini, and the tight nucleosome structure represses the transcription of the DNA by inhibiting the binding of general transcription factors to the gene (Jenster *et al*., 1997). Coactivators are proteins that enhance the transcriptional activation function of the nuclear receptors due to histone acetyltransferase activity and by providing bindings sites for other cofactors to form a stable preinitiation complex (Leo and Chen, 2000). There is a biological rationale, therefore, for the observed finding of an association between polymorphism in *NCOA*1 and some prolificacy traits in pigs

A second animal population supplied by Genex Swine Group, Canada, will be analyzed for the *NCOA*1-*Rsa*1 polymorphism. This population also consists of Meishan x Large White dam line nucleus herds. Both populations, Genex and Roslin crosses, will be analyzed for each parity for the polymorphism reported here and for others that may be identified during our further examination of the *NCOA*1 gene.

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