GENE EXPRESSION ANALYSIS IN ANTERIOR PITUITARY AND EST MAPPING TO INVESTIGATE GENETICS OF SWINE FERTILITY

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INTRODUCTION

The Nebraska (NE) selection lines originated from a White Composite base population. One line was maintained as the control (C) and a second was selected (I) for prolificacy. Selection was for an index of ovulation rate and embryonic survival (Generations 1 to 11) or litter size (Generations 12 to 16). The lines differed by 7.4 ova and 3.0 pigs per litter at Generation 14 (Johnson et al., 1999). In the present study, we investigated the effect of this selection on gene expression in anterior pituitary (AP).

Selection based on quantitative genetic theory results in allele frequency changes of many different genes having an effect on the trait being selected. However, the identity of these genes, and the frequencies and effects of their alleles in the selected population usually are not known. In the case of NE selection lines, the only information available is from a QTL study (Cassady et al., 2001), a candidate gene approach (Linville et al., 2001), and an ovarian follicle gene expression study (Gladney, 2000). After 16 generations of selection, the use of techniques that enable identification of differentially expressed genes provided us an unique opportunity to identify genes in AP, that possibly are associated with the pathway(s) that lead to phenotypic differences in these lines.

We hypothesized that selection quantitatively changed gene expression in AP as either direct or correlated responses. Differential display PCR (DD-PCR) was used to search for differences in gene expression between lines. Northern analysis was used to confirm DD-PCR results. In addition, the need to increase density in the swine mapping public data base led us to characterize the physical location of selected differentially expressed ESTs by using cytogenetic mapping (somatic cell hybrid panel; Yerle et al., 1996) and fine mapping (radiation hybrid panel; Yerle et al., 1998).

MATERIAL AND METHODS

A sample of 10 control lines sows and 13 select line sows of the 16th generation of the NE selection lines were used. The average of fully formed pigs in the second litter of these sows was 9.9 ± 2.02 for control sows and 12.46 ± 1.51 for index sows. In order to induce luteolysis and synchronize follicle development, sows from both lines were given an intramuscular injection of 2 mL of prostaglandin F₂α analog between days 12 and 14 of the estrous cycle. Tissues from sows from both lines were harvested two (D2) or four (D4) days after injection,
resulting in four different groups of sows: C-D2 (n = 6), C-D4 (n = 4), I-D2 (n = 6) and I-D4 (n = 7). For DD-PCR, two pools were produced from each line (C and I). Each pool had poly(A) RNA from one D2 sow and from one D4 sow resulting in four unique pools (Control line pools: CA-D2D4 and CB-D2'D4'; Index line pools: IC: I-D2D4 and ID: I-D2'D4'). Each pool had a concentration of 2.2 ng/µL of poly(A) RNA.

Differential display PCR (DD-PCR) was used to search for differences between lines in AP gene expression. Northern analysis was used to confirm DD-PCR results. The differential display of anterior pituitary tissue generated known, unknown, and novel EST sequences. A few previously unmapped ESTs having known biological functions were selected for cytogenetic and fine mapping. Primers were designed from EST sequence using Primer3 (Steve Rozen, Helen J. Skaletsky 1998, Primer3 ; Code available at: http://www-genome.wi.mit.edu/genome_software/other/primer3.html ) avoiding similarity to rodent sequence in the 3´ end. Most of the primers were designed in the 3´ UTR (untranslated region). In two instances (TCP1 and FTH) only one primer was designed in the 3´ UTR and in two other instances (Clock and SPARCL1) primers were designed within coding sequence.

RESULTS AND DISCUSSION
Weights of anterior pituitaries were significantly different (P = 0.0038) between control (351.0 mg) and index line (453.8 mg); whereas no difference was observed between weights of AP removed at day two (407.4 mg) and day four (410.9 mg) after injection of prostaglandin analog. The possible biological significance of the line difference in weight of AP is not known.

A total of 372 bands were extracted from the differential display gels, and prioritized for analysis based on visual inspection. Background of the lanes, consistency within pools of each line, and intensity of the differences between lines were considered in the prioritization to generate scores ranging from 1 to 6. All bands scored as 6 (n = 68) and 5 (n = 100) were cloned and sequenced. The majority of the bands were extracted from the index line (n = 246; 66.1%) amplification products. Some bands with similar expression between lines (n = 11) were cloned and sequenced to identify equally expressed genes for quality control.

Northern blot analyses were conducted for G-beta like protein, ferritin heavy-chain, and follicle stimulation hormone beta subunit. The differences and direction of expression of these genes observed in the differential display was confirmed using Northern blot analysis. Selection decreased expression in two of the genes analyzed (G-beta like protein and ferritin heavy-chain) and increased expression of follicle stimulation hormone beta subunit.

The pooling of RNA used in the present experiment has advantages and disadvantages. Our goal was to emphasize differences between lines regarding mRNA level. It was expected that the pooling strategy would reduce differences in mRNA level due to variation among animals within a line. Thus a disadvantage of using pools is that the true variability of the mRNA levels within a line is not well represented. However, despite this disadvantage, the strategy of using pooling enabled the identification of genes differentially expressed between lines.
It is not possible to determine whether the genes differentially expressed harbor polymorphisms responsible for the phenotypic differences between lines (i.e. QTL). It is likely that these differences are correlated responses to selection for the upstream QTL. Linkage studies using the expression profiles of these genes as phenotypic traits, and association studies of the differentially expressed genes, are required to further investigate whether the differentially expressed genes represent QTL upon which selection has changed allelic frequencies, or correlated responses resulting from QTL effects.

The present report includes mapping of ESTs on nine different pig chromosomes (Table 1). SCHP mapping allowed regional assignment of markers to chromosomes, and RH mapping refined mapping location and defined marker order not resolved in the SCHP map of the pig. This is important for identifying genes in QTL regions, in applying marker-assisted selection, and enhancing comparative maps with human and pigs. Increasing the density of markers in the porcine radiation hybrid map and better characterizing regions of lower marker density are fundamental aspects to merging genetic and physical maps, and thus assigning positional candidate genes for QTLs. In the present study for instance, the radiation hybrid mapping of SPARCL1 enabled the assignment of this EST within the QTL region for age at puberty previously identified in the NE selection lines (Cassady et al., 2001), thus identifying SPARCL1 as a positional candidate gene for age at puberty in these lines.

Table 1. Mapped EST symbol, accession number, cytogenetic assignment in the somatic hybrid cell panel (SHCP), correlation of mapped marker with the assigned physical position \( (R^2) \), chromosome position assigned using the radiation hybrid panel (RHP), marker linked and LOD score of the linkage between mapped marker and markers previously assigned to the pig RH map are presented below.

<table>
<thead>
<tr>
<th>SYMBOL</th>
<th>ACC. #</th>
<th>CHROMOSOME POSITION PIG</th>
<th>SCHP</th>
<th>R²</th>
<th>MARKER</th>
<th>LOD</th>
</tr>
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<tbody>
<tr>
<td>SPARCL1</td>
<td>BE344576</td>
<td>8q23-q27</td>
<td>0.8528</td>
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<td>SSP1</td>
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<tr>
<td>ATF4</td>
<td>BE344562</td>
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<td>0.8605</td>
<td>5</td>
<td>AC02</td>
<td>7.78</td>
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<tr>
<td>MEF2C</td>
<td>BE344561</td>
<td>2(1/2 q21)-(1/2 q22)</td>
<td>0.8478</td>
<td>2</td>
<td>SW2134</td>
<td>13.26</td>
</tr>
<tr>
<td>FTH</td>
<td>BE344556</td>
<td>2p14-p17</td>
<td>0.8748</td>
<td>2</td>
<td>SWR783</td>
<td>18.42</td>
</tr>
<tr>
<td>FRAP2</td>
<td>BE344575</td>
<td>6q22-q23</td>
<td>1.0000</td>
<td>6</td>
<td>SW1355</td>
<td>18.39</td>
</tr>
<tr>
<td>PBP</td>
<td>BE344574</td>
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<td>0.8748</td>
<td>14</td>
<td>SW2508</td>
<td>13.42</td>
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<td>STMEM*</td>
<td>BE344552</td>
<td>Xq22</td>
<td>0.8367</td>
<td>X</td>
<td>SW1943</td>
<td>5.35</td>
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<tr>
<td>LOC92004</td>
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<td>13q23-(1/2 q41)</td>
<td>1.0000</td>
<td>13</td>
<td>CP</td>
<td>8.51</td>
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<tr>
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<td>12p11-(2/3 p13)</td>
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<td>1</td>
<td>SW1824</td>
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<tr>
<td>SF3B1</td>
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<td>15q23-q26</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Clock</td>
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<td>8q11-q12</td>
<td>0.6860</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
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</table>

* Symbol applied only for this study; \( R^2 \) Correlation coefficient; ACC, # : Accession number
CONCLUSION
We have identified several changes in gene expression in the anterior pituitary as a result of long-term selection to increase ovulation rate and reduce embryo mortality. While this work demonstrates that genetic selection for complex, polygenic characters leads to measurable changes in transcription of potentially important genes, we cannot determine if these genes represent direct (QTL) or correlated (physiologically downstream) responses to selection. This work also contributes to the characterization of the transcriptome of the anterior pituitary of pigs during the follicular development phase, and to the association of function of these transcripts with fertility.

ACKNOWLEDGEMENTS
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REFERENCES