THE PROLACTIN RECEPTOR GENE IS ASSOCIATED WITH INCREASED LITTER SIZE IN PIGS

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SUMMARY

The prolactin receptor gene was investigated as a candidate gene for reproduction traits in five PIC lines consisting of Large White (2), Landrace, Duroc, and Large White/Meishan origin. Least square means for total number born and number born alive were calculated for each genotype in addition to an analysis of additive and dominance effects by line. The gene was significantly associated with total number born and/or number born alive in three of the lines tested. The magnitude ranged from 0.66 to greater than 1 pig per litter increase between homozygous genotypes and varied with background genetics. Also of interest was the lack of effect on average birth weight with the increase in litter size. This gene test has the potential to be a powerful tool when used in conjunction with traditional selection methods for some lines. Additional data is needed to confirm the significant effects seen in the three lines.

Keywords: Pig, prolactin receptor, marker assisted selection, candidate genes

INTRODUCTION

Improvement of reproductive traits in livestock species has become of increasing interest, especially in swine where moderate increases in litter size can equal large gains in profit. Marker assisted selection (MAS) could potentially be employed in conjunction with traditional selection methods to accelerate the rate of change in economically important traits. Two methods to identify markers linked to these quantitative traits are genomic scans and the candidate gene approach. In order to detect quantitative trait loci (QTL) using genomic scans, a locus with a moderate to large effect and large population sizes are required. There have been limited studies of this kind done for reproductive traits. Using the candidate gene approach, the estrogen receptor gene (*ESR*) was investigated and has been demonstrated to have large allelic effects ranging from 0.4 to 1.15 pigs per litter increase in the pig (Rothschild *et al.* 1996; Short *et al.* 1997). Other hormone receptors are presumed to be good candidate genes for quantitative traits because they are the limiting steps in many reproductive pathways.

Prolactin (PRL) is an anterior pituitary peptide hormone essential for reproductive success. Its receptor (PRLR) has been detected in various tissues including brain, ovary, placenta, and uterus in several mammalian species (Kelly *et al.* 1991). Mice homozygous for null mutations in PRLR are sterile due to a failure of embryonic implantation and also demonstrate irregular cycles, reduced fertilization rates, and defective embryonic development (Ormandy *et al.*

1997). PRLR numbers in luteal cells increase during pregnancy in the pig (Jammes *et al.* 1985). The *PRLR* gene has been recently linkage and physically mapped to pig chromosome 16 (Vincent *et al.* 1997). These characteristics make *PRLR* a strong candidate gene for reproductive traits.

MATERIALS AND METHODS

A total of 2,714 litter records from 1,077 sows were included in the litter size analyses. Traits included total number born (TNB) and number born alive (NBA) from five different PIC lines. The five lines examined were of Large White (two different origins) and Landrace origin, as well as synthetic lines consisting of ¾ Duroc, ¼ Large White, and Large White/Meishan origin. These lines were all housed in genetic nucleus farms owned by PIC U. K. or PIC U. S. and were raised in accordance with approved farm management practices. Animals were genotyped for the *PRLR* marker at Dalgety Food Technology Center (Cambridge, UK), PIC's Genetic Diagnostic Laboratory (Franklin, KY), or Iowa State University.

DNA was extracted from blood or tail tissue. Blood was collected in 50mM EDTA at pH 8.0 and tails were collected at birth and stored at -20°C. Fifty µl of blood was dispensed into a 0.5ml tube and 450µl of Tris-EDTA buffer was added to lyse the red blood cells. These tubes were vortexed for 2 seconds and then centrifuged for 12 seconds at 13,000g in a microcentrifuge. The supernatant was removed by aspiration. An additional 450µl TE buffer was added to the pellet and the procedure repeated until no traces of red blood cells could be seen in the pellet. The white blood cell pellet or the tail tissue was suspended in 100µl of a proteinase K buffer and the mixture incubated at 55°C for 2 hours. The samples were then incubated at 95°C for 8 min and the DNA lysates stored at -20°C. The PRLR fragment was amplified from genomic template using the polymerase chain reaction (PCR). Each 25µl reaction contained 2mM $MgCl_2$, 1X PCR buffer, .2mM dNTP, .4µM each primer, .6 unit Taq polymerase, and 20ng DNA. The primers used were forward primer: 5'-CCC AAA ACA GCA GGA GAA CG-3' and reverse primer: 5'-GGC AAG TGG TTG AAA ATG GA-3'. The reaction conditions were 93°C for 3 minutes, 35 cycles of 93°C for 30 seconds, 60°C for 1 minute, 72°C for 1 minute, and a final extension at 72°C for 3 minutes. Twenty µl of the PCR product was digested with 4 units *AluI* in 1X NEBuffer 2 (New England Biolabs) at 37°C overnight. The fragments were separated on a 6% NuSieve gel (FMC) and stained with Ethidium Bromide. The 90 base pair fragment was designated the A allele, and the 110 base pair fragment was designated the B allele.

The TNB and NBA traits were analyzed with a sire model including fixed effects of herd-season, service type (natural or AI), *PRLR* genotype, and parity. Sire was included as a random effect. *ESR* genotype was included as a fixed variable or a covariable. Interactions among herd, *ESR*, and *PRLR* were tested for significance. Heritability for the litter traits was assumed as .10 and repeatability as .21. Allele substitution effects were estimated by substituting for *PRLR* genotype a covariate which included the number of A alleles present. Dominance effects were estimated as the deviation of the heterozygotes from the average of the homozygotes.

RESULTS

The *PRLR* genotype was shown to explain a significant variation in litter size in three of the

lines tested. Two of the lines did not show any significant effect (P>0.1, results not shown). The least square means for TNB and NBA for each of the three significant lines are summarized in Table 1. The Large White based line sample consisted of 400 sows with 1197 litter records. The AA animals have a 0.66 pig per litter advantage in NBA over the AB and BB animals (P<0.05). There are indications of a dominance effect with the B allele. The Meishan based line sample consisted of 261 sows with 832 litter records. An additive effect for TNB (P<0.05) and NBA (P<0.05) and an overdominance effect for NBA (P<0.01) were observed in this line. The largest effect was detected in the Landrace based line sample, composed of 416 sows and 685 litter records. A greater than one pig per litter difference between the two homozygous genotypes was detected for both TNB (P<0.08) and NBA (P<0.1), with the A allele being favorable. There was no significant difference between genotypes for average birth weight in any lines tested.

Table 1. Least square means for each PRLR genotype across all parities for TNB and NBA for three commercial lines of pigs.

Commercial line	PRLR genotype	TNB	NBA
Large White Synthetic	AA	12.51	12.39
	AB	12.35	11.73
	BB	12.71	11.73
			P< 0.05
Effects	a	0.10	-0.33[b]
	d	-0.26	-0.33[a]
Meishan Synthetic	AA	13.64	12.95
	AB	14.35	13.74
	BB	13.96	13.27
		P< 0.05	P< 0.05
Effects	a	0.16[b]	0.16[b]
	d	0.55[b]	0.63[c]
Landrace Synthetic	AA	12.13	11.33
	AB	11.72	10.92
	BB	10.98	10.31
		P< 0.08	P< 0.10
Effects	a	0.51[b]	0.47[b]
	d	0.17	0.10

a=additive effect; d=dominance effect; effects are significant at [a]P<0.1, [b]P<0.05, [c]P<0.01;

DISCUSSION

PRLR was investigated as a candidate gene for litter size due to its integral role in several reproductive pathways. The results of this initial study indicate that *PRLR* has a significant effect on litter size as measured by TNB and NBA in three commercial lines. It is apparent that the background genetics of each different line plays a part in the manner and the magnitude that the trait is affected. The increased litter size in combination with no change in average birth weight is also an important aspect of the *PRLR* effect. Gains made by increased litter size

may be outweighed by losses due to smaller birth weights since smaller birth weights are correlated with decreased survivability. Although there was no difference shown for average birth weight, the use of *PRLR* must proceed with caution until further data can be collected and analyzed to assure no negative pleiotrophic effects exist with this and other traits. It is not clear at this time whether *PRLR* is a major gene for litter size or if it is linked to the gene having the effect. *PRLR* is mapped to chromosome 16 in the pig, which has a relatively low density of mapped genes for comparative analysis. Further mapping and investigation of genes or markers near *PRLR* are required to resolve this issue. Increasing the numbers of animals and records for each line will be required to confirm the effect. As experienced with the *ESR* gene, at least 1,000 sow records were necessary to reach a stable litter size effect of 0.4 pigs per litter (Short *et al.* 1997).

Incorporating reliable markers into a MAS program has potential for large financial returns in a shorter time frame due to a relatively low heritability and the sex limited nature of litter size. Selection can also be done before animals reach reproductive maturity. The early *PRLR* results indicate a sizable increase in number born alive in both the white dam lines. The existence of the gene association with litter size in the Landrace based line is especially useful since the favorable *ESR* B allele was in extremely low frequency. Additional markers such as *PRLR* give commercial breeders an alternative to marker assisted introgression of the B allele of the *ESR* gene from the Meishan for increasing litter size in lines having very low frequency of the favorable allele. The addition of *PRLR* in PIC's selection program allows lines previously excluded from MAS to be included and may be used in conjunction with the *ESR* test to further enhance selection efficiency.

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