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Study on the Prolactin Receptor 3 (PRLR3) Gene and the Retinol-binding Protein 4 (RBP4) Gene as Candidate Genes for Production Traits in Berkshire Pigs

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ABSTRACT : To investigate the influence of the prolactin receptor 3 (*PRLR3*) gene and the retinol-binding protein 4 (*RBP4*) gene on the production traits of swine, genotyping was performed on 156 and 141 Berkshire pigs, respectively, that were carefully selected for economic traits. The frequencies of allele *A* in the *PRLR3* locus and allele *B* in the *RBP4* locus were 0.50 and 0.42, respectively. Neither locus was in the Hardy-Weinberg equilibrium. After a genotype was assigned to the individuals whose parents had the homozygous genotype, a statistical analysis was conducted for 291 pigs. The animals with the *PRLR3* and *RBP4* genotypes included 182 and 227 head, respectively. Even though the genotypic effects of *PRLR3* ($p<0.05$) and *RBP4* ($p<0.01$) had a significant influence on the pigs' back fat thickness, the interaction of both genes was not highly significant in terms of the back fat thickness ($p = 0.1235$). While the estimated epistasis effects of *aaBB* and *aaBb* decreased the back fat thickness and reduced the growth rate, the effects of *AAbb* and *aabb* increased the growth rate. Despite the insignificant difference in the *PRLR* genotypes in terms of the days to 90 kg and the average daily gain, the back fat thickness showed a significant difference ($p<0.05$), and the additive effect of allele *A* and the dominant effect of the hetero-genotype were -0.377 and 1.206 mm, respectively. The *RBP4* genotypes had a very significant effect ($p<0.01$) on the back fat thickness, the days to 90 kg, and the average daily gain. The additive effects of allele *B* of the *RBP4* locus on the back fat thickness, the days to 90 kg, and the average daily gain were 0.70 mm, -1.3 days and 6.2 g, respectively. Moreover, the dominant effects of the heterozygote for those traits were 0.63 mm, 9.9 days and -45.0 g, respectively. Allele *A* of the *PRLR3* locus favorably influenced the back fat thickness, the days to 90 kg of the body weight, and the average daily gain and its dominant effect unfavorably influenced those traits. Allele *B* of *RBP4* showed an incremental growth rate and back fat thickness, which could lower the lean meat percentage in the carcass. The *RBP4* hetero-genotype negatively affected the pork production. These results strongly imply that the selection of allele A of *PRLR3* and allele *B* of *RBP4* would produce highly productive pigs in the Berkshire breed. Careful selection of allele B of *RBP4* is required because of the increase in the back fat thickness. (**Key Words :** Prolactin Receptor, Retinol-binding Protein, Candidate Gene, Additive Effect, Production Traits)

INTRODUCTION

The pig population in Korea is mainly composed of highly productive breeds such as the Landrace, Yorkshire and Duroc breeds and their crossbreeds. Berkshire pigs are raised for quality pork, and are publicly known for the quality of their carcass. Niche markets have been reported (Honeyman et al., 2006) for Berkshire pork, including from internet sales, local abattoir sales, direct marketing, farmer networks and targeting of organized groups in the U.S. Even in Korea, the meat of Berkshire pigs is sold as high-quality pork in supermarkets, with a premium price. A study found Berkshire-sired pigs superior in terms of most of their eating quality traits, such as their cooking loss and tenderness (Mabry and Baas, 1998). The productivity of the Berkshire breed is not efficient, unlike other major breeds. Small litter sizes were also observed in Berkshire breeds (Do, 2007b). Mabry and Baas (1998) reported that Berkshire-sired pigs had the most fat and the small loin muscle areas. It was because of this that increasing productivity came to the attention of Berkshire farmers.

Marker-assisted selection (MAS) by genetic markers is a tool to improve swine productivity. Genes such as

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Table 1. Classification of animals by *PRLR3* and *RBP4* genotype

	PRLR3				*RBP4*			
	AA	*Aa*	*aa*	Total	*BB*	*Bb*	*bb*	Total
Genotyped	22 (36)	101 (72)	21 (36)	144	34 (24)	54 (74)	68 (58)	156
Assigned[1]	0	160	35	195	62	118	138	318
Total	22	261	56	339	96	172	206	474
Unknown[2]	3	77	39	119	57	76	121	254

The figures in the parenthesis represent the expected numbers of animals under the Hardy-Weinberg Equilibrium.
[1] Represents the animals genotyped by parent information.
[2] Represents the numbers of animals which do not have information of genotype in other gene.

melanocortin 4 receptor (*MC4R*) (Kim et al., 2000) for growth, ryanodine receptor (*RYR1*) (Fujii, 1991), and heart fatty acid binding protein (*HFABP*) (Gerbens et al., 1999) for meat quality have been identified in pigs as associated with economic traits.

Prolactin receptor (*PRLR*), which mediates the signal transduction pathway in target endocrine tissues (Bole-Feysot et al., 1998; Goffin et al., 2002), has been shown to play certain roles in inducing milk-protein gene expression in the mammary gland (Rui et al., 1992). Retinol-binding proteins (*RBP*s), which are the specific carriers of retinol (vitamin A alcohol) in the blood, deliver retinol from the liver to the peripheral tissues. In pigs, alleles for the *PRLR* and *RBP4* genes have been associated with significant differences in litter size (Vincent et al., 1998; Rothschild et al., 2000; Drogemuller et al., 2001) and in fetus and early growth (Do et al., 2010).

Production traits, such as back fat thickness, days to 90 kg, and average daily gain, are also vital elements of the revenue of pig farmers. To properly practice MAS using the candidate genes without economic loss, the association of the genes with meat production traits should be considered. In this study, the influence and characteristics of *PRLR3* and *RBP4* genes on the back fat thickness, days to 90 kg, and average daily gain of Berkshire pigs were examined.

MATERIALS AND METHODS

Animals and DNA isolation

The Berkshire pigs were subjected to intense selection of their production and reproduction traits over six generations. During this period, approximately 20 boars and 100 sows were continuously raised in the herd. Computer breeding software was used to minimize inbreeding and to augment genetic enhancement of economic traits. Accordingly, the final inbreeding coefficient was estimated to have been approximately 1.6%. Genotyping was performed on 339 and 474 animals to characterize *PRLR3* and *RBP4*, respectively, as shown in Table 1. The classification and least square means of the pigs with growth records were also determined and are summarized in Table 2 and 3. Some of the male piglets were castrated on day 2 or 3. There were 291 genotyped animals with records of back fat thickness, days to 90 kg, and average daily gain, which were measured at 156.8 days (standard deviation: 11.7 days) of age and adjusted using the growth curves for the Berkshire breed (Do, 2007a). The genomic DNA was isolated from the blood samples of the pigs using the Toyobo MagExtraction Kit.

Primer design and polymerase chain reaction

The *PRLR3* and *RBP4* genes of the Berkshire pigs were

Table 2. Distribution of production traits by genotype, birth year and gender

Birth year		Gender		Parity		*PRLR3*		*RBP4*	
2003	35	Female	203	1	93	*AA*	14	*BB*	41
2004	62	Male	72	2	62	*Aa*	131	*Bb*	75
2005	124	Castrated	16	3	32	*Aa*	37	*Bb*	111
2006	70			4	25				
				5	25				
				6	25				
				7	16				
				≥8	13				
Total	291		291		291		182		227

Table 3. Least square means of back fat thickness, days to 90 kg and average daily gain

	Gender			Parity			
	Back fat thickness (mm)	Days to 90 kg	Average daily gain (g)		Back fat thickness (mm)	Days to 90 kg	Average daily gain (g)
Female	17.034±0.282	156.75±1.63	575.4±6.6	1	14.809±0.432	147.84±2.46	619.1±10.3
Male	16.577±0.388	150.00±2.25	611.4±9.1	2	17.445±0.479	149.60±2.73	608.0±11.4
Castrated	18.070±0.755	148.65±4.38	614.3±17.7	3	17.012±0.693	149.51±3.95	608.9±16.5
				4	16.949±0.964	175.87±5.49	510.6±22.9
				5	16.613±1.009	148.61±5.75	608.9±24.0
				6	14.887±1.453	155.75±8.28	577.5±34.6
				7	16.242±1.301	178.48±7.41	503.5±30.9
				≥8	22.477±1.997	149.50±11.38	601.0±47.5

amplified and obtained using the following primer pairs:

PRLR3: Forward 5'-CGT GGC TCC GTT TGA AGA ACC-3'

Reverse 5'-CTG AAA GGA GTG CAT AAA GCC-3'

RBP4: Forward 5'-GAG CAA GAT GGA ATG GGT T-3'

Reverse 5'-CTC GGT GTC TGT AAA GGT G-3'

PCR was performed in a 10 μl reaction mixture that contained 12 ng of genomic DNA, 10 pmol of the primer, 200 μM of dNTP, 2.5 units of *Taq* DNA polymerase (Enzynomics^{TM}, Korea), and the reaction buffer with 1.5 mM of $MgCl_2$. The reaction was carried out using a PTC-200 thermocycler (MJ Research, Watertown, MA, USA) with 5-min primary denaturation at 94°C, 45 s at the annealing temperature, 60 s at 72°C and a final 10-min extension at 72°C.

Polymorphism identification and genotyping

The polymorphic sites were tested for restriction fragment length polymorphisms (RFLPs) according to the NEBcutter program after each DNA sample from the Berkshire breed was genotyped. All the restriction enzymes were purchased from New England BioLabs (NEB) (Ipswich, MA, USA), and the restriction digestions were performed according to Rothschild et al. (2000).

The PCR product was incubated with 8U *Alu I* (NEB) and electrophoresed on a 3% Metaphor (FMC) agarose gel to generate several fragments. The combination of 85-bp, 59-bp and 19-bp represented the *AA* genotype, and the 104-bp and 59-bp fragments represented the *BB* genotype. The digestion of the remaining PCR product was performed with 4 U of *MspI*, and the fragments were resolved on 3% FMC gel such that the 190-bp, 154-bp and 136-bp fragments were used to observe *AA*, and the 154-bp, 136-bp and 125-bp fragments were used to observe *BB*.

Statistical analysis

The GLM procedure of SAS (2001) was used to assess the effects of the genotype. The data were analyzed by birth year, sex and dam's parity, along with the genotype of the candidate genes. The epistasis effects were calculated from the deviation of the least square means of *PRLR3***RBP4*, *PRLR3* and *RBP4* from the population mean of the model. The epistasis effect of *AA*/*BB* was the difference of *AA*/*BB* from the sum of *AA* and *BB* (Karain et al., 1979). To assess the additive effects, the least square means of the two homozygous genotypes were compared. The dominance effects were calculated on the basis of the deviation of the heterozygote effect from the mean of the two homozygous genotypes.

RESULTS AND DISCUSSION

Polymorphisms were observed in the *PRLR3* and *RBP4* loci of the Berkshire pigs. The Hardy-Weinberg equilibrium was checked with the number of animals that were genotyped, and the expected numbers are shown in Table 1. The frequencies of allele A in the *PRLR3* locus and allele *B* in the *RBP4* locus were 0.50 and 0.42, respectively. Neither locus showed the typical Hardy-Weinberg equilibrium. The frequencies of the hetero-genotypes were higher in the *PRLR3* gene and lower in the *RBP4* gene than the expected frequency that was obtained based on the Hardy-Weinberg principle. The genotype was assigned to offspring whose parents were homozygote, without further genotyping in a laboratory. The counts of the animals that were genotyped by pedigree information were 195 and 318 for *PRLR3* and *RBP4*, respectively. The number of genotyped animals with production records for each gene differed due to the difference in the number of animals genotyped, as shown in Table 2.

Pigs with generally less back fat, fewer days to gain 90 kg of body weight, and higher daily growth gains are required to increase a farmers revenue. The traits of days to 90 kg and average daily gain are closely related, as they indicate how fast a pig grows. The data on these two traits

Table 4. Analysis of variance for back fat thickness

Source	df	MS	F	df	MS	F	df	MS	F
Year	3	228.93	48.23 [a]	3	192.75	28.20 [a]	3	141.74	25.84 [a]
Gender	2	40.50	8.53 [a]	2	41.44	6.06 [a]	2	34.19	6.23 [a]
Parity	7	34.14	7.19 [a]	8	16.67	2.44 [b]	6	12.52	2.28 [b]
PRLR3	2	20.03	4.22 [b]						
RBP4				2	35.71	5.23 [a]			
*PRLR3*RBP4*							8	8.99	1.64 [d]
Error	167	4.75		211	6.83		98	5.48	

[a] p<0.01; [b] p<0.05; [d] p<0.25.

were negatively correlated, though. The basic statistics for those production traits are shown in Table 3. The pigs that were castrated at birth showed higher back fat thickness and average daily gain than the other pigs, but lower days to 90 kg. The number of animals in Table 2 decreased rapidly in closer parity with the dam, because the sows' reproductive traits were selected. The birth year, gender and dam parity were included in the models to eliminate their effects when the genotypic effects were estimated. These were considered environmental influences on the phenotype of the traits and hence, not relevant to transmittable genetic ability.

Statistical epistasis is a population property, and is a function of both the allele frequencies and the biological interactions among genes (Carter et al., 2005). The analysis of gene interaction characterizes whether or not multiple genes influence a particular genetic trait. It is not certain if two or more genes can interact to express a particular phenotype. Multiple gene products can also contribute to the expression of a single phenotype along the biochemical pathways in cells (Klug et al., 2007). Even though the genotypic effects of *PRLR3* (p<0.05) and *RBP4* (p<0.01) were significant in terms of the back fat thickness, the interaction of both genes was not significant in terms of the back fat thickness (p = 0.1235). The estimated epistatic effects of *aaBB* and *aaBb* were negative, at -1.516 and -1.514 mm, respectively. This extent of thickness reduction due to epistasis could lure animal breeders to further investigate large animal populations for MAS applications. The interactions of the genes were very significant (p<0.01) in the traits of days to 90 kg and average daily gain. This may strongly imply the presence of epistasis between the *RBP4* and *PRLR3* genes. The genotypes of *aaBB* and *aaBb* with a reduction in the back fat thickness also showed a decrease in the growth rate traits of days to 90 kg and average daily gain. The epistatic effects of the *AAbb* and *aabb* genotypes increased the rate of growth by -8.2 and -5.8 days for 90 kg of body weight and by 42.0 and 27.8 g for daily gain, respectively.

Despite the insignificant difference between the PRLR genotypes in terms of days to 90 kg and average daily gain (Tables 5 and 6), the back fat thickness showed a significant difference (p<0.05), as seen in Table 4. The additive effect of allele A was -0.377 mm in terms of back fat thickness, as shown in Table 8. This may strongly imply that the PRLR gene negatively affects the synthesis or deposition of subcutaneous fat. The positive medium genetic correlation (0.24) (Do, 2007b) of the back fat thickness with the litter size in Berkshire pigs and the significant allelic substitution effect (0.71 piglets in litter size with allele a) (Drogemuller et al., 2001) of *PRLR3* indicates an apparent relationship between the *PRLR3* gene and the back fat thickness. Though no significant days to 90 kg and average daily gain according to the genotypes of *PRLR3* were shown, the estimated additive effects were -4.54 days and 18.5 g, respectively. This appears to support the results of Freemark

Table 5. Analysis of variance for days to 90 kg

Source	df	MS	F value	df	MS	F	df	MS	F
Year	3	5241.1	25.94 [a]	3	2,641.6	13.09 [a]	3	1689.0	8.85 [a]
Gender	2	753.3	3.73 [b]	2	606.0	3.00 [c]	2	380.5	1.99 [d]
Parity	7	1,060.0	5.25 [a]	8	293.4	1.45 [d]	6	459.1	2.41 [b]
PRLR3	2	118.5	0.59						
RBP4				2	1,622.2	8.04 [a]			
*PRLR3*RBP4*							8	539.8	2.83 [a]
Error	167	202.1		211	201.8		98	190.8	

[a] p<0.01; [b] p<0.05; [c] p<0.10; [d] p<0.25.

Table 6. Analysis of variance for average daily gain

Source	df	MS	F	df	MS	F	df	MS	F
Year	3	104,461.0	30.71 [a]	3	49,335.9	14.86 [a]	3	33,061.7	10.57 [a]
Gender	2	22,264.4	6.55 [a]	2	15,881.9	4.79 [a]	2	10,159.6	3.25 [b]
Parity	7	13,782.0	4.05 [a]	8	5,880.7	1.77 [c]	6	6,707.7	2.14 [c]
PRLR3	2	2,066.7	0.61						
RBP4				2	33,314.9	10.04 [a]			
*PRLR3*RBP4*							8	11,157.2	3.57 [a]
Error	167	3,401.3		211	3,319.0		98	3,128.3	

[a] $p<0.01$; [b] $p<0.05$; [c] $p<0.10$.

et al. (2001) that the absence of PRLRs in mice was accompanied by reduced body weight. The dominant effect of the *PRLR3* genotype was demonstrated by 1.206 mm of back fat thickness, as shown in Table 8.

*RBP*s have been known to play important roles in maintaining visual function and have additional importance related to Vitamin A concerning growth in mammals. West et al. (1997) reported the relationship of Vitamin A to child growth. The genotypes of *RBP4* showed a very significant effect ($p<0.01$) on the back fat thickness, days to 90 kg, and average daily gain, as shown in Table 4, 5 and 6. The least square means of genotypes *BB*, *Bb* and *bb* in the *RBP4* gene were 17.83, 17.76 and 16.42 mm for the back fat thickness, 145.1, 156.3 and 147.6 days for the days to 90 kg and 628.1, 576.9 and 615.7 g for the average daily gain, respectively. Accordingly, the additive effects of *B* allele for these traits were 0.70 mm, -1.3 days and 6.2 g, respectively. Unlike the reduction of the back fat thickness by allele *A* of the *PRLR3* locus, allele B of the *RBP4* locus showed a consistent increase in the growth rate and the back fat thickness. Despite the unfavorable impact of allele *B* of the *RBP4* gene on the back fat thickness, it could reduce the number of days to reach 90 kg by 1.26 days or increase the growth rate by 6.2 g per day. This was inconsistent with the lower feeder weight (age: 74 days) of the Berkshire pigs (data not shown), and the *RBP4* gene possibly produced different growth curves according to the genotype. The dominant effects of the heterozygote for those traits were 0.63 mm, 9.9 days and -45.0 g, respectively.

Table 7. Epistatic effects of *PRLR3*/*RBP4* genes[1]

Traits	*PRLR3*	*RBP4*		
		BB	*Bb*	*bb*
Back fat thickness (mm)	*AA*	-0.490	1.523	-0.530
	Aa	0.605	0.235	0.189
	aa	-1.516	-1.540	1.525
Days to 90 kg	*AA*	5.267	1.259	-8.167
	Aa	-4.146	-2.170	1.454
	aa	9.328	2.928	-5.754
Average daily gain (g)	*AA*	-24.399	-8.271	41.981
	Aa	19.258	10.360	-7.537
	aa	-44.412	-14.800	27.820

[1] All estimates of least square means for obtaining epistatic effects were highly significant ($p<0.0001$).

In summary, this genetic study investigated the significance of the prolactin receptor 3 (*PRLR3*) and the retinol-binding protein 4 (*RBP4*) genes in the meat production traits of Berkshire pigs. Allele A of the *PRLR3* locus favorably influenced the back fat thickness, days to a 90 kg body weight and average daily gain, and its dominant effect unfavorably influenced these traits. Allele *B* of *RBP4* showed an increased growth rate and a higher back fat thickness, which could lower the lean meat percentage of the carcass. The hetero-genotype of *RBP4* negatively

Table 8. Additive and dominant (d) genetic effects of *PRLR3* and *RBP4* genes in production traits[1]

		Back fat thickness (mm)	Days to 90 kg	Average daily gain (g)
PRLR3	*AA*	15.221±0.729	150.36±4.75	609.4±19.5
	Aa	16.803±0.398	154.66±2.59	591.3±10.7
	aa	15.974±0.546	154.90±3.56	590.9±14.6
	AA-aa	-0.753	-4.54	18.5
	d	1.206	2.03	-8.9
RBP4	*BB*	17.832±0.580	145.12±3.15	628.1±12.8
	Bb	17.763±0.523	156.30±2.84	576.9±11.5
	bb	16.423±0.534	147.63±2.90	615.7±11.8
	BB-bb	1.409	-2.51	12.4
	d	0.634	9.93	-45.0

[1] All estimates of least square means were highly significant ($p<0.0001$).

affected the pork production. These results strongly imply that the selection of allele A of *PRLR3* and allele B of *RBP4* would result in more productive Berkshire pigs.

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