Journal of Animal Science and Technology 53(3) 185~193, 2011
DOI:10.5187/JAST.2011.53.3.185

Characterization of the Prolactin Receptor 3 (*PRLR3*) and Retinol-Binding Protein 4 (*RBP4*) Genes in the Birth Weight and Early Growth of Berkshire Pigs

ChangHee Do[1], DongHee Lee[2] and ByungWook Cho[3]*

[1]Department of Animal Biosystem Sciences, College of Agriculture and Life Sciences, Chungnam National University, Yuseong, Daejeon 305-764, Korea [2]Department of Life Science, University of Seoul, 13 Siripdae-kil, Dongdaemun-ku 130-743, Korea [3]College of Natural Resource and Life Sciences, Pusan National University, Miryang, 627-706, Korea

ABSTRACT

To investigate the influences of candidate genes on the birth weight and the early stages of life, genotyping of the prolactin receptor 3 (*PRLR3*) and retinol-binding protein 4 (*RBP4*) genes was performed in 156 and 141 Berkshire pigs, respectively. The frequency of both *PRLR3* alleles *A* and *a* was 0.50. The frequencies of the *RBP4* alleles *B* and *b* were 0.42 and 0.58, respectively. Neither locus was in Hardy-Weinberg equilibrium. No significant associations of the *PRLR3* alleles with birth or weaning weights and of the *RBP4* alleles with birth weight were observed. The proportions of the phenotype variances due to the genotypes of *PRLR3* in the feeder weights was 4.0% and those of *RBP4* in the weaning and feeder weights were 11.9 and 3.3%, respectively ($P < 0.05$). The dominance effect of *PRLR3* and *RBP4* on feeder weights was 2.40 and −1.86 kg, respectively ($P < 0.01$). The additive and dominance effects of *RBP4* on weaning weights were 0.332 and −0.682 kg, respectively ($P < 0.01$). Even if no significant epistasis of *PRLR3* and *RBP4* was detected, a considerable trend of consistent positive epistasis estimates of *AA/BB* and *Aa/Bb* was observed for all traits. The results of this study may have a considerable impact on early-stage growth by both loci, and a selection strategy should be designed separately for each marker in Berkshire pigs.
(**Key words :** Prolactin receptor, Retinol-binding protein, Candidate genes)

INTRODUCTION

The physiological actions of prolactin (PRL) are mediated by its transmembrane receptor (PRLR), a member of the cytokine receptor superfamily that mediates signal transduction pathways via a series of physiological events in target endocrine tissues (Bole-Feysot et al., 1998 Goffin et al., 2002). PRLRs have been shown to play an important role in inducing the expression of the milk protein gene in the mammary gland (Rui et al., 1992). The levels of PRL-binding sites vary during puberty (Maes et al., 1983), pregnancy, and lactation (Jahn et al., 1991; Buck et al., 1992). Consequently, the PRLR has significant influences on female fertility and lactation. Retinol-binding proteins (RBPs) belong to the lipocalin family and are the specific carriers of retinol (vitamin A alcohol) in the blood. RBPs also deliver retinol from the liver to the peripheral tissues. Among the RBP genes, *RBP4* is known to be highly expressed, especially during pregnancy, in pigs (Harney et al., 1993). Brief and Chew (1985) showed that supplementing the diet of pregnant sows with vitamin A can increase the litter size of farrowing pigs. In addition, West et al. (1997) reported that vitamin A influenced the growth of children.

Using candidate gene analysis, several major genes have been identified to be associated with the economically important traits of pigs. In pigs, alleles for the *PRLR3* and *RBP4* genes have been associated with significant differences in litter size (Vincent et al., 1998; Rothschild et al., 2000). In terms of body weight, birth weight decreases as litter size increases (Johnson et al., 1999) and heavy birth weight is important for lifetime performance (Rydhmer et al., 1989). Because growth at early stages of life was expected to be influenced by these genetic compounds, the influences of the *PRLR3* and *RBP4* genes on the birth weight and the body weights of 2.5-month-old Berkshire pigs were examined in this study.

* Corresponding author : ByungWook Cho, College of Natural Resource and Life Sciences, Pusan National University, Miryang, Gyeongnam 627-706, Korea. Tel: 82-55-350-5510, Fax: 82-55-350-5519, Email: bwcho@pusan.ac.kr

MATERIALS AND METHODS

1. Animals and DNA Isolation

From 2003 to 2007, 5 generations of Berkshire pigs were reared under the condition of intense selection for their productive and reproductive traits at Gyungnam Swine Research Institute (Sancheong, South Korea). About 20 boars and 100 sows were maintained in the herd during this period. A computerized mating program was used to prevent high levels of inbreeding and to augment the genetic improvement of economically important traits. Consequently, the average inbreeding coefficient was estimated to be approximately 1.6%. The numbers of the animals in the herd genotyped for the *PRLR3* and *RBP4* genes were 339 and 474, respectively, as shown in Table 1.

The distribution and least square means of the animals with growth records are shown in Table 1. Some male piglets were castrated on day 2 or 3. The weaning weight and feeder weight were measured after 23.2 days (SD: 3.3 days) and 74.0 days (SD: 5.1 days), respectively. Blood samples were collected from the pigs for DNA isolation by using the Toyobo MagExtraction Kit (Toyobo, Japan).

2. Primer Design for Polymerase Chain Reaction

Two primer sets were analyzed forrestriction fragment length polymorphism (RFLP) genotyping of the 2 candidate genes (*PRLR3* and *RBP4*). Fragments of the *PRLR3* and *RBP4* genes were amplified 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' and *RBP4*:

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

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

Polymerase chain reactions were performed in 10 μl volumes containing 12 ng of genomic DNA, 10 pmol of each primer, 200 μM of each dNTP, 2.5 units of *Taq* DNA polymerase (Enzynomics^{TM}, Korea), and reaction buffer with 1.5 mM $MgCl_2$. Thermal cycling reactions were performed using PTC-200 thermocycler (MJ Research, Watertown, MA, USA) for 5 min at 94℃ for an initial denaturation, followed by 30 cycles of 60 sec at 94℃ for denaturation, 45 sec at 55℃ for annealing, 60 sec at 72℃ for primer extension, and a final extension of 10 min at 72℃.

3. Polymorphism Identification and Genotyping

The polymorphic sites were analyzed for RFLPs using the NEBcutter program (New England Biolabs, Ipswich, MA, USA). Genotyping was performed on individual DNA samples from the Berkshire breed. All the restriction enzymes were purchased from New England BioLabs (N.E.B.), and the restriction digestions were performed as previously described (Rothschild et al., 2000).

The PCR product was digested with 8U *Alu I* (N.E.B.) and separated on a 3% Metaphor (FMC BioProducts, Rockland, MN, USA) agarose gel such that 85 bp, 59 bp, and 19 bp fragments were observed for the *AA* genotype and 104 bp and 59 bp fragments for the *BB* genotype. The remaining PCR product was digested with 4U of *MspI*, and the fragments were separated on a 3% Metaphor (FMC) gel to allow 190 bp, 154 bp, and 136 bp fragments to be observed for the *AA* genotype, and 154 bp, 136 bp, and 125 bp fragments for the *BB* genotype.

Table 1. Least square means (±SE) of the birth, weaning, and feeder weights (kg) by gender and parity

Gender	N	Birth weight	Weaning weight	Feeder weight	Parity	N	Birth weight	Weaning weight	Feeder weight
Female	240	1.40 (0.03)	6.8 (0.2)	25.5 (0.6)	1	113	1.35 (0.05)	6.3 (0.2)	29.3 (0.8)
Male	98	1.44 (0.04)	6.9 (0.2)	26.2 (0.7)	2	103	1.33 (0.05)	6.0 (0.2)	26.1 (0.8)
Castrate	121		6.8 (0.2)	25.8 (0.7)	3	65	1.41 (0.05)	6.9 (0.3)	25.9 (1.0)
					4	41	1.40 (0.07)	7.1 (0.3)	25.6 (1.2)
					5	42	1.26 (0.06)	6.4 (0.3)	26.4 (1.1)
					6	39	1.44 (0.07)	7.5 (0.3)	25.3 (1.2)
					7	27	1.48 (0.08)	5.6 (0.4)	23.6 (1.5)
					≥8	29	1.65 (0.08)	6.7 (0.4)	24.2 (1.6)

4. Statistical Analysis

Hardy-Weinberg equilibrium was tested by comparing the expected and observed genotype frequencies using a chi-square test. A linear model was employed for the association analyses between the genotypes of the marker loci and the phenotypic traits. The 2 marker loci and each trait were analyzed separately. The birth, weaning, and feeder weights were analyzed using the GLM and MIXED procedures of SAS (Cary, NC, USA) employing the following linear animal model:

$$\mathbf{y} = YS_i + G_j + DP_k + A + GT_l + e_{ijkl},$$

where **y** is the vector of observations for the traits, YS_i is the contemporary group, **i** is the i^{th} birth year-month for farrowing, $\mathbf{G_j}$ is the gender of the animal, $\mathbf{DP_k}$ is the parity number of the dam, A is a covariate for age at the time of measurement (except for birth weight), and $\mathbf{GT_m}$ is the marker genotype of the individual. In addition, random effects included a residual effect (e_{ijkl}). To ensure that there were enough observations in the contemporary group, the seasons of farrowing within a year were classified as spring (March-May), summer (June-August), fall (September-November), and winter (December-February). The additive genetic effects of the candidate genes were estimated via a pair-wise comparison of the least square means of the 2 homozygous genotypes, and the dominance effects were calculated as the deviation of the heterozygote effect from the average of the 2 homozygous genotypes. The probability for *F*-test of estimated additive and dominance effects was obtained by the linear contrast optionof the GLM procedure. Further, variance components of all factors except for the covariate of age measured by days were estimated by the MIXED procedure of SAS with the same model used for treating all of the effects (except for the age covariate in days) as random in order to investigate the proportion of variance due to the genotypes of the marker loci.

An additional model was employed to estimate the specific combining ability as follows:

$$y = YS_i + G_j + DP_k + A + P_l + R_m + PR_{lm} + e_{ijkl},$$

where P_l is the effects of genotype l in the *PRLR3* locus, R_m is the effects of genotype m in the *RBP4* locus, and PR_{lm} is the effect of their interaction. The specific combining ability was calculated from the deviation of genotypes for each locus and their interactions, as follows:

$$S_{ij} = (I_{ij} - I..) - (A_i - A.) - (B_i - B.)$$

where S_{ij} is the specific combining ability of genotype i of the *A* locus and genotype j of the *B* locus, I_{ij} is the least square mean of the interaction for genotype i of the *A* locus and genotype j of the *B* locus, and A_i and B_i are the least square mean of the i and j genotypes of the *A* and *B* loci, respectively.

RESULTS

The statistics on the basic traits of the Berkshire pigs are shown in Table 1. Even though the male pigs that were castrated at birth showed a higher average daily weight gain than the other pigs (Do, 2007), no significant differences were seen in the birth, weaning, and feeder weights of the pigs. The offspring from the second parity weighed less at birth and during weaning and feeding than the first parity.

Variance components analyzed by the MIXED procedure and *F*-statistics analyzed by the GLM procedure of SAS is presented in Tables 2 and 3. For feeder weight, the observed

Table 2. Variance components and *F*-statistics of the birth, weaning and feeder weights for the prolactin receptor 3 (*PRLR3*) gene

Source	df[1)]	Birth weight			Weaning weight			Feeder weight		
		VC[2)]	%[2)]	*F*[3)]	VC	%	*F*	VC	%	*F*
Year-season	11	0.0252	25.8	4.86[a]	0.436	20.4	5.72[a]	24.13	52.5	13.05[a]
Gender	2	0.0031	3.2	8.48[a]	0.025	1.2	1.97[d]	1.05	2.3	6.03[a]
Parity	7	0.0158	16.1	6.72[a]	0.403	18.9	5.33[a]	0.68	1.5	1.79[c]
Age days[4)]	1						49.99[a]			34.49[a]
PRLR3	2	0.0000	0.0	0.77[e]	0.000	0.0	0.34[e]	1.83	4.0	6.67[a]
Residual	228	0.0537	54.9		1.271	59.5		18.26	39.7	
Phenotype		0.0978			2.135			45.95		

[1)] represents df for the records of weaning weight; [2)] represents variance components (VC) and % when all effects except for the covariate of age (in days) were treated as random; [3)] represents *F*-statistics when all effects were treated as fixed; [4)] represents age of days at measurement.
[a] $P < 0.01$; [b] $P < 0.05$; [c] $P < 0.10$; [d] $P < 0.20$; [e] $P > 0.20$.

Table 3. Variance components and *F*-statistics of the birth, weaning and feeder weights for retinol-binding protein 4 (*RBP4*) gene

Source	df[1]	Birth weight			Weaning weight			Feeder weight		
		VC[2]	%[2]	*F*[3]	VC	%	*F*	VC	%	*F*
Year-season	13	0.0359	34.5	10.26[a]	0.312	14.2	4.94[a]	8.08	27.4	7.80[a]
Gender	2	0.0004	0.4	2.23[d]	0.001	0.1	0.60[e]	0.00	0.0	0.66[e]
Parity	8	0.0042	4.0	2.98[a]	0.155	7.1	4.19[a]	0.00	0.0	0.64[e]
Age days[4]	1						40.51[a]			28.60[a]
RBP4	2	0.0002	0.0	1.52[e]	0.263	11.9	10.63[a]	0.96	3.3	3.90[b]
Residual	334	0.0633	60.8		1.468	66.8		20.46	69.3	
Phenotype		0.1040			2.199			29.50		

[1] represents df for the records of weaning weight; [2] represents variance component (VC) and portion from phenotype variance, respectively, when all effects except for the covariate of age (in days) were treated as random; [3] represents *F*-statistics when all effects were treated as fixed; [4] represents age of days at measurement.
[a] $P < 0.01$; [b] $P < 0.05$; [c] $P < 0.10$; [d] $P < 0.20$; [e] $P > 0.20$.

variance due to the genotype of *PRLR3* was 1.83, which is 4.0% of the phenotype variance, and this was significant at $P > 0.01$ with the F-test, as shown in Table 2. However, the differences with respect to the *PRLR3* genotype were not significant ($P > 0.05$) for the birth and weaning weights. The variations due to the genotype of *RBP4* are presented in Table 3. For the birth weight, the variance component due to the genotype of the locus was 0.0002, which was small and not significant ($P > 0.05$). The differences in the weaning and feeder weights with respect to *RBP4* genotype, however, were significant ($P < 0.01$ and $P < 0.05$, respectively). Further, the portions of the phenotype variance of weaning weight and feeder weight due to the *RBP4* genotype were 11.9 and 3.3%, respectively, which implies that *RBP4* is a potential candidate gene that is important for the early growth of Berkshire pigs. However, an impact of 1 locus on a trait that accounts for over 10% of the phenotype variance does not seem to be realistic, considering that quantitative traits are usually expressed by polygenes. Therefore, unknown quantitative trait loci that have an effect on the growth of pigs may be linked to these genes, or, alternatively, the effects of the genotype could be confounded with unknown environmental factors. Under the circumstances, the variation due to these genotypes could therefore be overestimated.

The frequency of the animals according to the genotypes of the marker loci is given in Table 4. The *PRLR3* and *RBP4* loci showed polymorphismsin the Berkshire herd. The Hardy-Weinberg equilibrium was checked for the animals genotyped in the laboratory. The frequencies of the *A* and *a* alleles in the *PRLR3* locus were both 0.50. The frequencies of the *B* and *b* alleles in the *RBP4* locus were 0.42 and 0.58, respectively. When both parents were homozygotes, the genotype was assigned to their offspring without further genotyping in the laboratory. The numbers of the animals that were genotyped by pedigree information were 195 and 318 for *PRLR3* and *RBP4*, respectively. As shown in Table 4, the numbers of the genotyped animals with body weight records differed according to their traits due to missing data. For the *PRLR3* and *RBP4* genotypes, the frequencies significantly differed from the Hardy-Weinberg equilibrium ($P < 0.005$, using a chi-square test). The frequencies of the hetero-genotypes were higher than expected for the *PRLR3* gene, which is consistent with a synthetic line reported by Drogemuller et al (2001), and lower for the *RBP4* gene than expected based on the Hardy-Weinberg principle. Drogemuller et al (2001) had reported that the *PRLR3* and *RBP4* genotypes in a German Landrace and Duroc line were in Hardy-Weinberg equilibrium. Selection practice on a population with equilibrium causes disequilibrium (Falconer and Mackay, 1996). Dominance effects that are greater than the additive effects of body weights as shown in Table 5 would lead to selection of the heterozygote in the *PRLR3* locus, and thus, result in a higher frequency.

For the *PRLR3* and *RBP4* polymorphisms, genotypes and the additive and dominance effects are shown in Tables 4 and 5. The differences between the *RBP4* genotypes for birth weight were rather small (i.e., ranging from 0.014 to 0.114 kg in Table 4) and were not significant (Table 3). Further, the differences between the *PRLR3* genotypes were small (i.e., ranging from 0.019 to 0.048 kg, and from 0.011 to

Table 4. Classification of animals and number of records by prolactin receptor 3 (*PRLR3*) and retinol-binding protein 4 (*RBP4*) genotypes

	PRLR3				*RBP4*			
	AA	*Aa*	*aa*	Total	*BB*	*Bb*	*bb*	Total
Genotyped	22	101	21	144	34	54	68	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
No. records[3)]	15	186	51	232	76	120	165	361
LS means								
Birth weight	1.368	1.397	1.349		1.514	1.454	1.440	
Weaning weight	5.941	5.952	5.780		7.299	6.285	6.635	
Feeder weight	24.202	26.294	23.582		27.152	25.072	26.720	

[1)] represents the animals genotyped by parent information.
[2)] represents the number of animals that do not have information on the genotype of the other gene.
[3)] represents the number of records for weaning weight.

0.172 kg for the differences in the birth and weaning weights, respectively, in Table 4) and were not significant (Table 2). With respect to the feeder weight, the *AA* animals in *PRLR3* had an advantage of 0.62 kg over the *aa* animals (P (a = 0) = 0.63), resulting in 0.31 kg of an additive effect, and heterozygous animals had a size superiority (d = 2.401 kg; P < 0.01). Further, favorable additive effects of the *B* allele in the *RBP4* locus were detected for the feeder weight (0.216 kg), but the results of the association analyses showed no significant effects (P (a=0) = 0.59). The difference calculated between the heterozygote and the average of the two homozygous genotypes reached −1.864 kg (P < 0.01) for the feeder weight (Table 5). This may imply that the *PRLR3* gene has some impact on the growth of the individual itself but only after weaning.

The frequency of the heterozygote in the *PRLR3* gene was higher than expected, as shown in Table 4. Considering the significant positive dominance effect of the heterozygote on the feeder weight, the intense selection for a higher increment in the body weight could be a reason for the higher frequency of the heterozygote. Despite minimal impact on the birth weight, the genotypes of *RBP4* showed a highly significant effect on the weaning and feeder weights. The least square means of the genotypes *BB, Bb,* and *bb* in the *RBP4* gene were 7.299, 6.285, and 6.635 kg, respectively, for the weaning weight and 27.15, 25.07, and 26.72 kg, respectively, for the feeder weight. The largest effect was detected in the weaning weight of the *RBP4* genotypes, which was composed of 76 *BB*, 120 *Bb* and 165 *bb* records. A difference of 0.664 kg between the 2 homozygous genotypes was observed for the weaning weight (Table 4), resulting in a significant additive effect of the *RBP4* allele *B*, in which the favorable allelic substitution effect was 0.332 kg (P < 0.01) (Table 5). The additive effect of the *B* allele on the feeder weights was 0.216 kg. The dominance effects of the heterozygote on the birth, weaning, and feeder weights were −0.023, −0.682, and −1.864 kg, respectively. Thus, the selection of the *B* allele would contribute to a

Table 5. Additive (a) effects (kg) of the prolactin receptor 3 (*PRLR3*) gene allele *A* over *a* and retinol-binding protein 4 (*RBP4*) gene allele *B* over *b* and dominance (d) effects (kg) with respect to birth, weaning, and feeder weights

	PRLR3				*RBP4*			
	a	P (a=0)	d	P (d=0)	a	P (a=0)	d	P (d=0)
Birth weight	0.009	0.79	0.040	0.34	0.037	0.09	−0.023	0.53
Weaning eight	0.081	0.65	0.091	0.66	0.332	0.00[1)]	−0.682	0.00[1)]
Feeder weight	0.310	0.63	2.401	0.00[1)]	0.216	0.59	−1.864	0.00[1)]

[1)] indicates P < 0.01.

higher body weight, which can result in decreased mortality (Danish Veterinary and Food Administration, 2009).

Specific combining ability is usually used to estimate interactions in a two-factorial experiment or epistasis in a genetic experiment (Soltan et al., 1977). Even if accurate estimates of epistasis are not available, the estimates of the specific combining ability allow us to conjecture the epistasis effects with zero overall means. The genotype effects of the *PRLR3* and *RBP4* loci and the effects of their interactions were included in the model with the same fixed effects as the previous genotype model in order to estimate the specific combining ability. Only 3 or 4 significant comparisons out of a total of 24 comparisons for each trait were detected in the linear contrasts of the effects ($P < 0.05$). However, significances are not presented for the specific combining abilities shown in Table 6. The specific combining abilities of *AA/BB* of each trait were positive and gradually increased as pigs aged (e.g., 0.131, 0.32, and 3.2 kg for the birth, weaning, and feeder weights, respectively). In addition, the specific combining abilities of *Aa/Bb* of each trait were positive and gradually increased (e.g., 0.07, 0.4, and 1.7 kg for the birth, weaning, and feeder weights, respectively). On the other hand, the specific combining abilities of *aa/BB* and *Aa/bb* of each trait were negative and gradually decreased as pigs aged. Further, *AA/Bb* showed negative values for each trait. The epistasis effects for the rest of the genotypes showed inconsistency in whether their values were positive or negative. The number of records was not sufficient for obtaining significance in the epistasis analysis because of the number of factors being compared. However, there were trends that animals with *aa/BB* or *Aa/bb* genotypes had decreased body weights, and animals with *AA/BB* or *Aa/Bb* genotypes had increased body weights. However, the information obtained by the estimation of thespecific combining ability is limited in terms of accuracy, and further research is necessary in order to use epistasis in pig breeding.

DISCUSSION

Genetic markers allow the identification of animals carrying beneficial or harmful alleles early in life, thereby improving the accuracy, reducing the generation interval, and accelerating the genetic improvement of the trait. For example, selection based on DNA information in pigs was used to eliminate the deleterious *HAL n* allele (Fujii et al., 1991). As more associations between markers and traits are identified, this technology may be used by animal breeders in order to enhance genetic improvements.

Weight gain during the early stages of the pig life is related to viability (Danish Veterinary and Food Administration Factsheet, 2009). Further, a small birth weight has a negative influence on postnatal growth (De Passille et al., 1993; Klemcke et al., 1993) and piglet performance at early stages of growth is crucial for lifetime performance (Chimonyo et al., 2008).

Prolactin is a hormone that influences the reproduction and milk secretion of pigs (Goffin et al., 2002). According to Isler et al (2000), the *a* allele of *PRLR* positively influenced the number of fetuses per uterine horn. Larger litter sizes resulted in smallerindividuals in the litter, which can be explained by the negative genetic correlation between birth weight and the average size of piglets (Bérard et al., 2008). The results of initial studies indicated that the *PRLR* allele *A* had a significant effect on the number of piglets born alive, but unfortunately their estimates of the additive effects of the *A* allele fluctuated from −0.33 to +0.47 piglets per litter, and the mode of gene action was inconsistent (Vincent et al., 1998). Early growth of the pigs depends on the milk

Table 6. Specific combining ability[1)] (kg) for the genotypes of prolactin receptor 3 (*PRLR3*) and retinol-binding protein 4 (*RBP4*) genes

PRLR3	*RBP4*								
	Birth weight			Weaning weight			Feeder weight		
	BB	*Bb*	*bb*	*BB*	*Bb*	*bb*	*BB*	*Bb*	*bb*
AA	0.131	−0.202	0.071	0.32	−0.13	0.57	3.2	−2.5	−0.7
Aa	−0.024	0.070	−0.046	0.23	0.40	−0.08	0.1	1.7	−1.8
Aa	−0.107	0.132	−0.025	−1.59	−0.37	0.66	−3.2	0.8	2.4

[1)] estimated from comparisons of the least square means of *PRLR3* and *RBP4* genotypes and their interactions, and these comparisons were not statistically significant.

production of the dam, which is partially influenced by the *PRLR3* gene of the dam (Jahn et al., 1991 Buck et al., 1992). With the *PRLR* marker located some distance from the unknown quantitative trait locus, associations between the marker and the trait may vary between populations, lines, or families and this may be a possible reason for the lack of significant *PRLR* effects in the German Landrace line (Drogemuller et al., 2001).

In the current study, *PRLR3* was investigated as a potential candidate gene influencing the early growth of Berkshire pigs. The *A* allele at the *PRLR* locus had positive additive effects on prenatal and early stage growth. The observed contrasts in the birth and weaning weights among the genotypes of *PRLR3*, however, were found not to be significant. This may be due to the limited number of records examined in this study. Rothschild et al (2000), using data from nearly 2,800 records, reported a significant additive effect of + 0.15 in the number of pigs born alive associated with *RBP4*, while Drogemuller et al (2001) did not obtain a significant result for the same gene and traits with 1,037 records. Another possible reason for the lack of a *PRLR3* effect on prenatal and early growth of pigs in the current study is that no linkage disequilibrium existed. Consistently positive additive and dominance effects for the *PRLR3* locus, however, were seen in the prenatal and early growth stages of the pig, and this could be used to facilitate genetic improvement in the productivity of Berkshire pigs. For a confirmed result on *PRLR3*, further research with a sufficient number of records is necessary.

RBPs have been characterized as essential in maintaining visual function in many biochemical studies of mice and humans. *RBP*-deficient mice displayed reduced blood retinol levels and impaired visual function during the first months of life (Paik et al., 2004). Vitamin A has additionally been shown to be important for the growth of mammals. Retinol-binding protein is known to be a carrier of vitamin A, which is related to the litter size of pigs (Rothschild et al., 2000) and the growth of children (West et al., 1997). Negative dominance effects on the growth of pigs in the current study were evident, and these were stronger than the additive effects. The *B* allele, mentioned as favorable by Rothschild et al (2000), had a lower frequency than the alternative allele. Further, the hetero-genotype of *RBP4* had a lower frequency than the homo-genotypes. This may suggest selection against the hetero-genotype. However, the positive additive effects with significance for the birth ($P < 0.10$) and weaning ($P < 0.01$) weights are important, considering the low frequency of the *B* allele. Selection for the homo-genotype of *BB* in order to avoid the negative dominance effect and unfavorable *b* allele would be an efficient way to improve the production capability of pigs.

The analysis of the gene interactionswas used to characterize if multiple genes influence a particular genetic trait. It does not indicate, however, if 2 or more genes interact with one another to express a particular phenotype. Rather, multiple gene products typically contribute to the expression of a single phenotype in cells (Klug et al., 2007). The development of an organ involves highly complex processes in order to produce a mature structure with multiple phenotypic manifestations. Thus, the birth weight and other traits may be influenced by the related individual activities of 2 genes during the development of the fetus and during early growth stages. There were some trends that the epistasis effects of *aa/BB, Aa/bb*, and *AA/Bb* were consistently negative, while those of *AA/BB* and *Aa/Bb* were consistently positive for the prenatal and early growth of pigs. This may imply the presence of an epistasis between the *RBP4* and *PRLR3* genes. If further research confirms influence on growth, a scheme for considering influence on the litter size of pigs by both markers should be developed for genetic improvement of pig growth.

The current genetic study characterized the significance of the *PRLR3* and *RBP4* genes in the birth weight and early growth of Berkshire pigs. The allele *A* of the *PRLR3* locus positively affected the birth weight and the early pig growth, and its dominanceeffect was positive. The allelic substitution effects of *RBP4* showed positive values on the body weights. The hetero-genotype of *RBP4* negatively affected the birth weight and the early growth of the pigs. The specific combining ability of double hetero-genotypes and of favorable double homo-genotypes of both *PRLR3* and *RBP4* were positive for the birth weight and early stage growth of the Berkshire pigs. Further, the stronger dominance effects compared to the additive effects were positive in *PRLR3* and negative in *RBP4*. Even if many of the non-significant results are taken into account, these results strongly imply that selection against the heterozygote of *RBP4* and for the heterozygote of *PRLR3* leads toa lower frequency in the hetero-genotypes of *RBP4* and a higher frequency in the hetero-genotypes of *PRLR3*, and are the genetic basis of the relatively heavy body weights in the Berkshire pigs of Korea. Also, selection for the *A* allele of the *PRLR3* locus

and the *BB* genotype of the *RBP4* locus should be considered.

The gene or loci of *PRLR3* and *RBP4* may not represent all the factors that are flanked, and possibly even controlled, by these loci. The two loci, however, have been well established as the prime markers in evaluating the litter size and growth, especially for this pig strain, in many articles (Harney et al., 1993; Isler et al., 2000; Rothschild et al., 2000). Further studies should be performed in order to investigate the effect or interaction of third factors or SNPs that flank the 2 genes in order to dissect out the genetic influences on the expression of the birth weight and early growth of Berkshire pigs. Allelic effects differ for each population due to their genetic background. Different linkage phases between the intronic markers and causal mutations due to recombination are always possible even in the different family in the same population. Furthermore, novel quantitative trait loci may be linked to these genes and may exert a significant effect on pig growth. These potential genetic complexities add difficulty in the analyses of candidate gene effects in the presentstudy.

ACKNOWLEDGMENT

This study was financially supported by the 2008 Intramural Research Fund of Chungnam National University.

REFERENCES

Bérard, J., Kreuzer, M. and Bee, G. 2008. Effect of litter size and birth weight on growth, carcass and pork quality, and their relationship to postmortem proteolysis. Journal of Animal Science 86, 2357-2368.

Bole-Feysot, C., Goffin, V., Edery, M., Binart, N. and Kelly, P. A. 1998. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocrine Review 19, 225-268.

Brief, S. and Chew, B. P. 1985. Effects of vitamin A and β-carotene on reproductive performance in gilts. Journal of Animal Science 60, 998-1004.

Buck, K., Vanek, M., Groner, B. and Ball, R. K. 1992. Multiple forms of prolactin receptor messenger ribonucleic acid are specifically expressed and regulated in murine tissues and the mammary cell line HC11. Endocrinology 130, 1108-1114.

Chimonyo, M., Dzama, K. and Bhebhe, E. 2008. Genetic determination of mothering ability and piglet growth in indigeneous Mukota sows of Zimbabwe. Livest. Sci. 113, 74-80.

Danish, Veterinary and Food Administration. 2009. Effects of Danish restrictions on non-therapeutic use of antibiotics. In: Danish Veterinary and Food Administration FACT sheet (12.08.2009), p2.

De Passille, AMB, Rushen, J. E., Foxcroft, G. R., Aherne, F. X. and Schaeffer, A. 1993. Performance of young pigs: Relationships with periparturient progesterone, prolactin and insulin of sows. J. Anim. Sci. 71:179-184.

Do, C. H. 2007. Estimation of growth traits using growth curve in Gyungnam-heugdon (Berkshire). Journal of Animal Science and Technology (Kor.) 49(2), 195-202.

Drogemuller, C., Hamann, H. and Distl, O. 2001. Candidate gene markers for litter size in different German pig lines. J. Anim. Sci. 79, 2565-2570.

Falconer, D. S. and Mackay, T. F. C. 1996. Introduction to quantitative genetics (4th edition), Pearson Prentice Hall, New York.

Fujii, J., Otsu, K., Zorzato, F., de Leon, S., Khanna, V. K., Weiler, J. E., O'Brien, P. J. and MacLennan, D. H. 1991. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science (Wash. DC) 253, 448-451.

Goffin, V., Binart, N., Touraine, P. and Kelly, P. A. 2002. Prolactin: the new biology of an old hormone. Annual Review of Physiology 64, 47-67.

Harney, J. P., Ott, T. L., Geisert, R. D. and Bazer, F. W. 1993. Retinol binding protein gene expression in cyclic and pregnant endometrium of pigs, sheep, and cattle. Biology of Reproduction 49. 1066-1073.

Isler, B. J., Ervin, K. M., Rothchild, M. F. and Evans, G. J. 2000. Association between the prolactine receptorgene and reproductive components in swine. In: Proceedings of the 27th International Conference of Animal Genetics (Minneapolis, MN), p 67.

Jahn, G. A., Edery, M., Belair, L., Kelly, P. A. and Djiane, J. 1991. Prolactin receptor gene expression in rat mammary gland and liver during pregnancy and lactation. Endocrinology 128, 2976-2984.

Johnson, R. K., Nielsen, M. K. and Casey, D. S. 1999. Responses in ovulation rate, embryonal survival, and litter traits in swine to 14 generations of selection to increase litter size. Journal of Animal Science 77, 541-557.

Klemcke, H. G., Lunstra, D. D., Brwon-Borg, H. M., Borg, K. E. and Christensen, R. K. 1993. Association between low birth

weight and adrenocortical function in neonatal pigs. J. Anim. Sci. 71:1010-1018.

Klug, W. S., Cummings, M. R. and Spencer, C. A. 2007. Essential of genetics. Pearson Prentice Hall, New Jersey.

Maes, M., Hertogh, R. D., Watrin-Granger, P. and Ketelslegers, J. M. 1983. Ontogeny of liver somatotropic and lactogenic binding sites in male and female rats. Endocrinology 113, 1325-1332.

Paik, J., Vogel, S., Quadro, L., Piantedosi, R., Gottesman, M., Lai, K., Hamberger, L., Vieira, M. and Blaner, W. S. 2004. Vitamin A: Overlapping delivery pathways to tissues from the circulation. Journal of Nutrition 134, 276S-280S.

Rothschild, M. F., Messer, L., Day, A., Wales, R., Short, T., Southwood, O. and Plastow, G. 2000. Investigation of the retinol-binding protein 4 (*RBP4*) gene as a candidate gene for increased litter size in pigs. Mammalian Genome 11, 75-77.

Rui, H., Djeu, J. Y., Evans, G. A., Kelly, P. A. and Farrar, W. L. 1992. Prolactin receptor triggering. Journal of Biological Chemistry 267, 24076-24081.

Rydhmer, L., Eliasson, L., Stern, S., Andersson, K. and Einarsson, S. 1989. Effects of piglet weight and fraternity size on performance, puberty and farrowing results. Acta Agric. Scand. 39, 397-406.

Soltan, M., Abdou, F. H., Abd-Ellatif, M. and Ayoub, H. 1977. Estimation of general and specific combining abilities from a diallel cross of three inbred lines of Fayoumi chicks. Ann. Genet. Sel. Anim. 9(3):387-391.

Vincent, A. L., Evans, G., Short, T. H., Southwood, O. I., Plastow, G. S., Tuggle, C. K. and Rothschild, M. F. 1998. The prolactin receptor gene is associated with increased litter size in pigs. In: Proceedings of the 6th World Congress of Genetic Application of Livestock Production Armidale, NSW, Australia. 27, 15-18.

West, K. P., LeClerq, S. C., Shrestha, S. R., Wu, LS-F., Pradhan, E. K., Khatry, S. K., Katz, J., Adhikari, R. and Sommer, A. 1997. Effects of Vitamin A on Growth of Vitamin A-Deficient Children: Field Studies in Nepal. Journal of Nutrition 127, 1957-1965.

(Received Jan. 11, 2011; Revised Mar. 9, 2011; Accepted Mar. 11, 2011)