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 targetting 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

**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. The additive effects of 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

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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.

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

| PRLR3 | RBP4 |
|-------|-------|
| AA    | BB    |
| Aa    | Bb    |
| aa    | bb    |

| Genotyped | Assigned¹ | Total |
|-----------|-----------|-------|
| 22 (36)   | 34 (24)   | 144   |
| 101 (72)  | 54 (74)   | 195   |
| 21 (36)   | 68 (58)   | 339   |
| Total     | 182       | 254   |

| Assigned² | Total |
|-----------|-------|
| 3 (24)    | 76    |
| 77 (24)   | 121   |
| 21 (36)   | 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.

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

| Birth year | Gender | PRLR3 | RBP4 |
|------------|--------|-------|------|
|            |        | AA    | BB   |
|            |        | Aa    | Bb   |
|            |        | aa    | bb   |

| Birth year | Gender | Parity | PRLR3 | RBP4 |
|------------|--------|--------|-------|------|
| 2003       | Female | 203    | 93    |      |
|            | Male   | 72     | 62    | 131  |
| 2005       | Castrated | 16 | 32    | 37   |
| 2006       |        | 4      | 25    |      |
|            |        | 5      | 25    |      |
|            |        | 6      | 25    |      |
|            |        | 7      | 16    |      |
|            |        | ≥8     | 13    |      |
| Total      | 291    | 182    | 227   |      |

The PRLR3 and RBP4 genes of the Berkshire pigs were
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 CA T AAA GCC-3'

**RBP4**: Forward 5'-GAG CAA GA T GGA A TG 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™, Korea), and the reaction buffer with 1.5 mM of MgCl₂. 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 Rothschild et al. (2000). The polymorphic sites were 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, and the expected numbers are shown in Table 1.

**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, and the expected numbers are shown in Table 1.

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 farmer’s 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

| Gender     | Back fat thickness (mm) | Days to 90 kg | Average daily gain (g) |
|------------|-------------------------|---------------|------------------------|
| Female     | 17.03±0.282             | 156.75±1.63   | 575.4±6.6              |
| Male       | 16.57±0.388             | 150.00±2.25   | 611.4±9.1              |
| Castrated  | 18.07±0.755             | 148.65±4.38   | 614.3±17.7             |

| Parity     | Back fat thickness (mm) | Days to 90 kg | Average daily gain (g) |
|------------|-------------------------|---------------|------------------------|
| 1          | 14.80±0.432             | 147.84±2.46   | 619.1±10.3             |
| 2          | 17.44±0.479             | 149.60±2.73   | 608.0±11.4             |
| 3          | 17.01±0.693             | 149.51±3.95   | 608.9±16.5             |
| 4          | 16.94±0.964             | 175.87±5.49   | 510.6±22.9             |
| 5          | 16.61±1.099             | 148.61±5.75   | 608.9±24.0             |
| 6          | 14.88±1.453             | 155.75±8.28   | 577.5±34.6             |
| 7          | 16.24±1.301             | 178.48±7.41   | 503.5±30.9             |
| ≥8         | 22.47±1.997             | 149.50±11.38  | 601.0±47.5             |
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 gene and the back fat thickness. The estimated epistatic effects 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 AAAb 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 4. Analysis of variance for back fat thickness

| Source       | df | MS       | F     | Source       | df | MS       | F     | Source       | df | MS       | F     |
|--------------|----|----------|-------|--------------|----|----------|-------|--------------|----|----------|-------|
| Year         | 3  | 228.93   | 48.23 | Gender       | 2  | 40.50    | 8.53  | Year         | 3  | 192.75   | 28.20 |
| Gender       | 2  | 40.50    | 8.53  | Parity       | 7  | 34.14    | 7.19  | Gender       | 2  | 41.44    | 6.06  |
| Parity       | 7  | 34.14    | 7.19  | PRLR3        | 2  | 20.03    | 4.22  | Parity       | 8  | 16.67    | 2.44  |
| PRLR3        | 2  | 20.03    | 4.22  | RBP4         | 2  | 35.71    | 5.23  | PRLR3*RBP4   |    |          |       |
| RBP4         |    |          |       | RBP4         |    |          |       | RBP4         |    |          |       |
| RBP4         |    |          |       | RBP4         |    |          |       | RBP4         |    |          |       |
| Error        | 167| 4.75     | 211   | Error        | 98 | 5.48     |       | Error        | 167| 202.1    |       |

\[p<0.01; \, p<0.05; \, p<0.10; \, p<0.25.\]

Table 5. Analysis of variance for days to 90 kg

| Source       | df | MS       | F value | Source       | df | MS       | F     | Source       | df | MS       | F     |
|--------------|----|----------|---------|--------------|----|----------|-------|--------------|----|----------|-------|
| Year         | 3  | 5241.1   | 25.94   | Gender       | 2  | 753.3    | 3.73  | Parity       | 7  | 1060.0   | 5.25  |
| Gender       | 2  | 753.3    | 3.73   | PRLR3        | 2  | 118.5    | 0.59  | RBP4         | 2  | 1622.2   | 8.04  |
| Parity       | 7  | 1060.0   | 5.25   | PRLR3*RBP4   |    |          |       | Error        | 167| 202.1    |       |
| PRLR3        | 2  | 118.5    | 0.59   | Error        | 211| 201.8    |       | Error        | 167| 202.1    |       |
| RBP4         |    |          |       | Error        | 98 | 190.8    |       | Error        | 167| 202.1    |       |

\[p<0.01; \, p<0.05; \, p<0.10; \, p<0.25.\]
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.

RBP4s 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 PRLR3 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, 678.0 and 615.7 g for the average daily gain, respectively. Unlike the allele for these traits were 0.70 mm, -1.3 days and 6.2 g, 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

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  | 3  | 49,335.9 | 14.86 | 3  | 33,061.7 | 10.57 |
| Gender       | 2  | 22,264.4  | 6.55  | 2  | 15,881.9 | 4.79  | 2  | 10,159.6 | 3.25  |
| Parity       | 7  | 13,782.0  | 4.05  | 8  | 5,880.7  | 1.77  | 6  | 6,707.7  | 2.14  |
| PRLR3*PRLR3  | 2  | 2,066.7   | 0.61  |     |         |      |     |         |      |
| PRLR3*RBP4   | 2  | 33,314.9  | 10.04 | 8  | 11,157.2 | 3.57  |     |         |      |
| Error        | 167| 3,401.3   |       | 211| 3,319.0  |       | 98 | 3,128.3  |      |

*p<0.01; *p<0.05; *p<0.10.

Table 7. Epistatic effects of PRLR3/RBP4 genes

| Traits      | PRLR3     | RBP4     |
|-------------|-----------|----------|
|             | BB        | Bb       | bb      |
| Back fat (mm) | AA       | -0.490   | 1.523   | -0.530   |
|             | Aa       | 0.605    | 0.235   | 0.189    |
| Days to 90 kg | AA       | -1.516   | -1.540  | 1.525    |
|             | Aa       | -4.146   | -2.170  | 1.454    |
| 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   |

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

effects of the heterozygote for those traits were 0.63 mm, 9.9 days and -45.0 g, respectively.

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

| PRLR3 | RBP4 |
|-------|------|
|       | Back fat thickness (mm) | Days to 90 kg | Average daily gain (g) |
| BB    | 17.83 ±0.580  | 145.12 ±3.15  | 628.1 ±12.8  |
| Bb    | 17.763 ±0.523 | 156.30 ±2.84  | 576.9 ±11.5  |
| BB-bb | 1.409     | -2.51         | 12.4         |
|       | 0.634     | -9.9          | -45.0        |

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 \textit{PRLR3} and allele B of \textit{RBP4} would result in more productive Berkshire pigs.

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