A Novel Single Nucleotide Polymorphism of the Leptin Receptor Gene Associated with Backfat Thickness in Duroc Pigs

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Received July 23, 2015 /Revised September 13, 2015 /Accepted September 21, 2015

Fatness is one of the most important economic traits in pigs. The leptin receptor (LEPR) gene may be a potential candidate for the fatness quantitative trait locus (QTL) on porcine chromosome 6, due to its position and physiological role. Thus, this study was carried out to evaluate the associations between structural variants in the LEPR gene and economic traits in pigs. We obtained an approximately 114-kb sequence containing the complete genomic DNA of the porcine LEPR gene, using shotgun sequencing of a bacterial artificial chromosome clone. We report the complete genomic structure of the porcine LEPR gene. Dozens of transcription factor-binding sites were found in the 1.2 kb upstream region from the transcription start point. An association study was performed with 550 Duroc pigs for 24 single-nucleotide polymorphisms (SNPs), including 6 SNPs within exons and 18 SNPs within the putative 5’ regulatory region of the porcine LEPR gene. Among them, one SNP (790C/G) was significantly associated with backfat thickness and lean meat percentage, whereas the others, including two SNPs with missense polymorphisms, had no effect on any phenotype. These results suggest that SNP 790C/G may be a useful marker for genetic improvements of fatness and leanness in Duroc pigs.

Key words: Economic trait, leptin receptor, pig, single nucleotide polymorphism

Introduction

Quantitative trait locus (QTL) mapping has been performed to detect chromosomal regions that are associated with production and meat quality traits by crossing phenotypically divergent breeds. To date, more than 6,800 QTLs representing 585 overlapping phenotypic traits have been deposited in pig QTDb (http://www.animalgenome.org/cgi-bin/QTLdb/SS/index). Moreover, several QTLs for growth and fat deposition traits have been identified in a similar region of swine chromosome (SSC) 6 [3, 6, 8, 11, 13, 17, 18, 25]. Subsequently, great efforts have been made to find causal mutations controlling the QTLs through fine mapping or positional candidate gene approaches. However, definite conclusions have not yet been drawn based on those results [1, 12, 19, 20, 25]. The leptin receptor (LEPR) gene is well known a potential positional candidate gene controlling QTL for growth and fatness traits in the long arm of SSC6 because of its position and biological function.

Leptin, produced primarily in adipose tissue, is involved in the regulation of feed intake, energy balance, and reproduction in mammals [5]. Leptin signaling is mediated via the LEPR, which belongs to the class I cytokine family [23]. Leptin and LEPR genetic variants are associated with obese phenotypes in humans and mice, and the two genes are expected to influence fat deposition in pigs [4]. Associations between LEPR variants and reproductive [2] and fatness traits [12] have been reported in pigs. Ovilo et al. (2005) found a significant association between LEPR alleles and backfat thickness in a narrow region (130 - 132 cm) of chromosome 6 [20]. In recent, Uemoto et al. (2012) detected a significant SNP (c.2002C>T) in exon 14 on fatness traits [24]. All association studies on LEPR have been performed between exonic or intronic mutations and phenotypes in pigs. A few cDNA sequences and partial sequences of the porcine LEPR have been deposited in GenBank (e.g., AF092422), but the complete genomic organization has not been characterized. Moreover, the 5’ regulatory region of the porcine LEPR gene sequence has not been published.

Therefore, this study was carried out to evaluate the por-
cine LEPR gene as a positional candidate controlling the QTL for growth and fat deposition traits on SSC6. In addition, we report the complete genomic structure containing the 5’ regulatory region of the porcine LEPR gene.

Materials and Methods

Ethics statement

The study protocol and standard operating procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the National Institute of Animal Science (No. 2009-077, C-grade).

Isolation of a bacterial artificial chromosome clone containing the porcine LEPR gene

A bacterial artificial chromosome (BAC) clone containing the LEPR gene was obtained from the Korean native pig (KNP) BAC library [10] using a polymerase chain reaction (PCR) screening method. A BAC clone containing the LEPR gene was screened with LEPR-CASTS (UniSTS: 253565, Forward: 5’-TTCCAGAAACATAAGACACGCG-3’, Reverse: 5’-GACCAATTCTAAATTTCAACCAGG-3’). A shotgun library of the screened BAC clone, KNP_645H8, was constructed using the pUC19 plasmid vector (Qbiogene, Irvine, CA, USA). The sequence was obtained using an ABI PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM3730 Genetic Analyzer (AppliedBiosystems). The DNA sequences were assembled with Phred and Phrap software (University of Washington). The assembled sequence was deposited into GenBank of NCBI (FN673752).

Structural analysis of the porcine LEPR gene

Exon-intron boundaries of the LEPR gene on the sequence of BAC clone were determined by comparing with the porcine mRNA sequence (AF092422). Potential transcription factor-binding sites in the 5’ upstream region were predicted using the TRANSFAC 8.4 professional program. The putative promoter sequence of the porcine LEPR gene was aligned with the human (AC097063) and mouse (AL929373) sequences using the ClustalW2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2/) to investigate consensus sequences within the promoter regions among species.

Single nucleotide polymorphism discovery

Single nucleotide polymorphisms (SNPs) within exons and a putative promoter region were detected by direct sequencing of the samples pooled from five different breeds, including the Korean native pig, Berkshire, Duroc, Landrace, and Yorkshire. Eleven pairs of primers covering 1.2 kb upstream and 11 exon regions were designed based on the BAC clone sequence obtained (Table 1). The PCR reaction was performed in a 50 μL final volume containing 50 ng template DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.5 mM MgCl₂, 0.2 μM each primer, 100 μM each dNTP, and one unit Taq DNA polymerase (GeNet Bio, Korea). Reaction profiles included a 5 min denaturation step at 94°C followed by 35 cycles each consisting of 30 s at 94°C, 30 s at the annealing temperature (Table 1), 1 min at 72°C, and then a final 10 min extension step at 72°C using a PTC-225 Peltier Thermal Cycler (MJ Research, Waltham MA, USA).

The PCR products were cleaned up with a QIAquick PCR purification Kit (Qiagen, Hilden, Germany) and sequenced with the respective PCR primers using BigDye Terminator Cycle Sequencing Kit version 3.2(Applied Biosystems, USA) and an 3730XL DNA Analyzer (AppliedBiosystems). SNPs were identified by multiple alignments of sequence chromatograms generated with each primer pair using SeqMan program of Lasergene package (DNASTAR, USA).

Genotyping and phenotypes

In total 1,014 pigs from nine different breeds including western breeds (Berkshire, Duroc, Landrace, and Large White), the Korean native pig, the Korean wild pig, and Chinese breeds (Xiang, Min, and Wuzhishan pig) were used to investigate the allelic frequencies of SNPs. The traits analyzed in this study were average daily weight gain, feed efficiency, backfat thickness, and lean meat percentage. Blood samples were collected from 550 Duroc pigs at the Pig Breeding Stock Evaluation Center of the Korean Swine Association in Korea for the association test. Genomic DNAs were extracted with the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). PCR reactions were performed in a 25 μl final volume containing 25 ng template DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.5 mM MgCl₂, 0.2 μM each primer, 100 μM each dNTP, and one unit Taq DNA polymerase (GeNet Bio) for genotyping of the 18 SNPs in the promoter region and the 6 SNPs in the exon region (Table 2). Thermal cycling parameters were defined as follows: pre-denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, annealing temperature for 30 s (Table 1), 72°C for 1 min, and then a final step at 72°C for 10 min.
Table 1. List of primer sequences used to amplify the porcine LEPR gene

| Primer name | Regions               | Sequences                                      | Product size (bp) | Annealing temp. |
|-------------|-----------------------|-----------------------------------------------|-------------------|----------------|
| 1FR         | 5UTR/Exon1/intron1    | ATGTAAAATAGCATTGCATGA TTACCCATTTAAAAGAAACAG   | 350               | 53             |
| 2FR         | Exon2/ intron2        | TGCATTGACTTGCCATATCC CAGTCTATATCAGCAGTGA     | 646               | 62             |
| 3FR         | intron3/Exon4/intron4 | AAGCTGGGTGGTCCAGAAGAG CAGGTTGTCACAAGTGAAGA  | 428               | 62             |
| 4FR         | intron4/Exon5/intron5 | ACCAATATTTGTTCGCCTGAA TCTTTAGCTGCTTCGAAT     | 354               | 55             |
| 5FR         | Intron6/Exon7/Intro7  | CAGCTCCAGTTGCTGACCTCTCCAGCG                  | 868               | 55             |
|             | /Exon8/intron8        | TCACCAATTAATCCCTCTGAA                        |                   |                |
| 6FR         | Intron11/Exon12/intron12 | TGGAAAAATATCTCCCTCAGAAGA GCGAGTCGACAAATGACTT | 232               | 54             |
| 7FR         | Intron12/Exon13/intron13 | GTCTTGATTCGGCAAGCTT GAAAGAGACCTTCCGACAC     | 466               | 62             |
| 8FR         | Intron13/exon14/intron14 | GCCCTTGTCAGTGAACAGCAG TACAGTGACCTCGGTTGAT     | 482               | 59             |
| 9FR         | Intron15/Exon16/intron16 | CCTAGTTGAGTGGTTTCTG AGAAATCTGACGGTTCCGAAT  | 265               | 54             |
| 10FR        | intron17/Exon18/3UTR  | CCCAGTTGATTTGTGACGACT GGAAGAGTTTGTACGGCGA    | 815               | 66             |
| proLEPR     | Upstream region       | TCCACCCAGTAAATTTTCA TACCCCAATGAAACAAAGCC     | 1205              | 53             |

Table 2. Exon-intron organization of the porcine LEPR gene

| Exon | cDNA (AF092422) | Exon size (bp) | Intron | Intron size (kb) | Exon | cDNA (AF092422) | Exon size (bp) | Intron | Intron size (kb) |
|------|----------------|----------------|--------|-----------------|------|----------------|----------------|--------|-----------------|
| 1    | 1-55           | 55             | 1      | 5.084           | 10   | 1619-1767      | 149            | 10     | 1.114           |
| 2    | 56-385         | 330            | 2      | 1.878           | 10   | 1768-1927      | 160            | 11     | 0.615           |
| 3    | 386-509        | 124            | 3      | 7.174           | 12   | 1928-2010      | 83             | 12     | 3.355           |
| 4    | 510-718        | 209            | 4      | 4.291           | 13   | 2011-2227      | 217            | 13     | 1.956           |
| 5    | 719-8864       | 146            | 5      | 2.573           | 14   | 2228-2410      | 183            | 14     | 1.667           |
| 6    | 864-1009       | 145            | 6      | 5.874           | 15   | 2411-2506      | 96             | 15     | 1.579           |
| 7    | 1010-1300      | 291            | 7      | 0.166           | 16   | 2507-2609      | 103            | 16     | 1.455           |
| 8    | 1301-1418      | 118            | 8      | 2.627           | 17   | 2610-2685      | 76             | 17     | 13.974          |
| 9    | 1419-1618      | 200            | 9      | 3.427           | 18   | 2686-4032      | 1347           |        |                 |

using a PTC 225 Peltier Thermal Cycler (MJ Research). Genotypes of 550 samples were determined by PCR-restriction fragment length polymorphism (PCR-RFLP) analyses using Tsp509I, HpycH4111, NdeI, AciI, DraIII, and Sau3AI (Table 2) for exonic mutations and direct sequencing for SNPs within the upstream region.

Statistical analysis
Data were analyzed with the general linear model procedure using SAS (SAS Institute, Cary, NC, USA) to test the effect of each genotype on performance traits. Mean differences were established based on the least squares means comparison. A p-value <0.05 was considered significant. The formula for analyzing traits was Y=Xβ+M+ε, where Y is the phenotype vector; X and β are the design matrix and solution vector for fixed effects including year/season of birth and gender and the performance testing period (days) covariate, respectively; M is a 3×1 vector of the genotype effects; and e is a residuals vector.
Table 3. Allelic frequencies of the porcine LEPR gene variants in nine pig breeds

| Breeds            | A223T (I73L) | A673G (T220A) | A744G (L243L) | C2187T (S775S) | A2340G (S775S) | C2885T (D947D) |
|-------------------|--------------|---------------|---------------|---------------|---------------|---------------|
|                    | A   | T   | A   | G   | A   | G   | C   | T   | A   | G   | C   | T   | A   | G   | C   | T   | A   | G   | C   | T   | A   | G   | C   | T   |
| Korean native pig | 24  | ND  | ND  | 0.15| 0.85| 0.85| 0.15| 0.50| 0.50| 0.55| 0.45| 0.45| 0.55 |
| Landrace          | 108 | 0.10| 0.90| 0.08| 0.92| 0.80| 0.20| 0.78| 0.22| 0.73| 0.27| 0.13| 0.87 |
| Duroc             | 550 | 0.20| 0.80| 0.00| 1.00| 0.68| 0.32| 0.71| 0.29| 0.66| 0.34| 0.22| 0.78 |
| Berkshire         | 77  | 0.09| 0.91| 0.09| 0.91| 0.87| 0.13| 0.69| 0.31| 0.75| 0.25| 0.08| 0.92 |
| Large White       | 182 | 0.02| 0.98| 0.27| 0.73| 0.99| 0.01| 0.43| 0.57| 0.52| 0.48| 0.26| 0.74 |
| Korean wild pig   | 19  | ND  | ND  | 0.16| 0.84| 1.00| 0.00| 0.81| 0.18| 0.65| 0.35| 0.36| 0.63 |
| Xiang             | 27  | ND  | ND  | 0.44| 0.56| 1.00| 0.00| 0.06| 0.94| 0.25| 0.75| 0.19| 0.81 |
| Min               | 7   | ND  | ND  | 0.33| 0.66| 1.00| 0.00| 0.08| 0.92| 0.45| 0.55| 0.31| 0.69 |
| Wuzhishan         | 20  | ND  | ND  | 0.65| 0.35| 1.00| 0.00| 0.15| 0.85| 0.58| 0.42| 0.69| 0.31 |

ND: Not determined
Table 4. SNPs position, allele frequencies and transcription factor binding sites of 5’ regulatory region of the porcine LEPR gene in Duroc breed

| Allele (1/2) | Allele frequency 1 | Allele frequency 2 | Transcription factor | Score | Sequence |
|------------|------------------|------------------|-------------------|-------|---------|
| -91 A/C    | 0.55             | 0.45             | -                 | -     | CATTTG  |
| -132 G/A   | 0.99             | 0.01             | EMF               | 100   | -       |
| -201 G/A   | 0.99             | 0.01             | -                 | -     | -       |
| -203 C/T   | 0.62             | 0.38             | GATA-1            | 100   | AATCT   |
| -230 A/T   | 0.99             | 0.01             | T3R-beta1         | 100   | AGTAA   |
| -303 T/C   | 0.90             | 0.10             | NF-AT             | 100   | GCCTT   |
| -324 A/C   | 0.91             | 0.09             | AP-1              | 100   | GTCTCA  |
| -378 A/G   | 0.99             | 0.01             | RXR-alpha         | 100   | TCCACC  |
| -468 C/T   | 0.90             | 0.10             | PTF1-beta         | 87.5  | CAGCTG  |
| -549 G/A   | 0.99             | 0.01             | AP-4              | 100   | GTA1CA  |
| -618 C/A   | 0.99             | 0.01             | C/EPBalpha        | 87.5  | ATGTGGAA |
| -681 C/T   | 0.91             | 0.09             | RXR-alpha         | 100   | AGATTA  |
| -698 A/G   | 0.91             | 0.09             | C/EPBalpha        | 87.5  | GTGGA   |
| -717 C/A   | 0.79             | 0.21             | STAT3             | 87.5  | AGTGGAAAT |
| -742 C/A   | 0.68             | 0.32             | TFID              | 100   | TCCAAA  |
| -761 C/T   | 0.78             | 0.22             | GR                | 100   | TCTTAT  |
| -790 C/G   | 0.71             | 0.29             | NF-kappaB         | 87.5  | AGCCAG  |
| -862 G/C   | 0.62             | 0.38             | -                 | -     | -       |

Allelic frequencies and association analysis

Allelic frequencies of these exonic polymorphisms were investigated in nine different pig breeds (Table 3). These polymorphisms were present in almost all breeds, except for the T220A polymorphism in Duroc and the L243L polymorphism in the Korean wild pig and the Chinese breeds. The allelic frequency of each locus showed different patterns among the pig breeds. Half of all SNPs in the 5’ regulatory region were not informative in Duroc (Table 4). Dozens of transcription factor-binding sites were predicted in the 5’ regulatory region of the porcine LEPR gene. Among them, specificity protein 1 and C/EBP sites have been found in the human leptin gene promoter [7], and C/EBPα and PPARγ modulate the expression of the human leptin gene [14, 21]. In addition, the transcription factors NFκB, liver X receptor, and hepatocyte nuclear factor-4α play important roles regulating gene expression of lipid metabolism [22].

A total of 550 pure Duroc pigs were genotyped on 24 SNPs including 6 SNPs on the exonic regions and 18 SNPs in the regulatory region for the association study. Only one SNP at the -790C/G polymorphism on the regulatory region in the LEPR gene was significantly associated with production traits such as backfat thickness ($p<0.001$) and lean
meat percentage (p<0.003), but had no significant effect on average daily weight gain or feed efficiency (Table 5). That is, backfat thickness was higher and lean meat percentage lower in the individual of the genotype GG rather than in that of the genotype CC. However, no other significant associations of genotypes for the other 24 SNPs were found for the other traits.

The -790C/G polymorphism site was generated within the binding site AGGACAC/GCC of the putative NF-kB transcription factor. NF-kB is involved in regulating gene expression as a transcription factor. This suggests that a polymorphism in the promoter region of the LEPR gene might be critical for binding a transcription factor such as NF-kB. A significant phenotypic effect may have been observed if a causal mutation in the LEPR promoter region occurred or if the mutation was closely linked with a causal mutation. Therefore, further studies are needed to determine whether these results are due to a polymorphic site that is critical for transcription or linkage disequilibrium with a causal mutation.

Acknowledgement

This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project title: Development of techniques and knowledge-database for applicable combination of genetic markers related to economic traits for Hanwoo and pig, Project No. PJ01022002)” Rural Development Administration, Republic of Korea.

References

1. Armyasi, M., Grinfeld, E., Javor, A. and Lien, S. 2006. Investigation of two candidate genes for meat quality traits in a quantitative trait locus region on SSC6: the porcine short heterodimer partner and heart fatty acid binding protein genes. J. Anim. Breed. Genet. 123, 198-203.
2. Chen, C. C., Chang, T. and Su, H. Y. 2004. Characterization of porcine leptin receptor polymorphisms and their association with reproduction and production traits. Anim. Biotechnol. 15, 89-102.
3. de Koning, D. J., Rattink, A. P., Harlizius, B., van Arendonk, J. A., Brascamp, E. W. and Groenen, M. A. 2000. Genome-wide scan for body composition in pigs reveals important role of imprinting. Proc. Natl. Acad. Sci. USA 97, 7947-7950.
4. Friedman, J. M. and Halaas, J. L. 1998. Leptin and the regulation of body weight in mammals. Nature 395, 762-770.
5. Friedman, J. M. 2002. The function of leptin in nutrition, weight, and physiology. Nutr. Rev. 60, 85-87.
6. Gerbers, F., de Koning, D. J., Harders, F. L., Meuwissen, T. H., Janes, L. L., Groenen, M. A., Veerkamp, J. H., Van Arendonk, J. A. and te Pas, M. F. 2000. The effect of adipocyte and heart fatty acid-binding protein genes on intramuscular fat and backfat content in Meishan crossbred pigs. J. Anim. Sci. 78, 552-559.
7. Gong, D. W., Bi, S., Pratley, R. E. and Weintraub, B. D. 1996. Genomic structure and promoter analysis of the human obese gene. J. Biol. Chem. 271, 3971-3974.
8. Grinfeld, E., Szyda, J., Liu, Z. and Lien, S. 2001. Detection of quantitative trait loci for meat quality in a commercial slaughter pig cross. Mamm. Genome 12, 299-304.
9. Jacob, M. and Gallinaro, H. 1989. The S’ splice site: phylogenetic evolution and variable geometry of association with U1RNA. Nucleic Acids Res. 11, 2159-2180.
10. Jeon, J. T., Park, E. W., Jeon, H. J., Kim, T. H., Lee, K. T. and Cheong, I. C. 2003. A large-insert porcine library with seven fold genome coverage: a tool for positional cloning of candidate genes for major quantitative traits. Mol. Cells 16, 113-116.
11. Kim, J. J., Rothschild, M. F., Beever, J., Rodriguez-Zas, S. and Dekkers, J. C. M. 2005. Joint analysis of two breed cross populations in pigs to improve detection and characterization of quantitative trait loci. J. Anim. Sci. 83, 1229-1240.
12. Mackowski, M., Szymoniak, K., Szydlowski, M., Kamczyc, M., Eckert, R., Rozyczki, M. and Siwulski, M. 2005. Missense mutations in exon 4 of the porcine LEPR gene encoding extracellular domain and their association with fatness traits. Anim. Genet. 36, 135-137.
13. Malek, M., Dekkers, J. C., Lee, H. K., Baas, T. J., Prusa, K., Huff-Lonergan, E. and Rothschild, M. F. 2001. A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. II. Meat and muscle
composition. Mamm. Genome 12, 637-645.
14. Miller, S. G., De Vos, P., Guerre-Millo, M., Wong, K., Hermann, T., Staels, B., Briggs, M. R. and Auwerx, J. 1996. The adipocyte specific transcription factor C/EBPalpha modulates human ob gene expression. Proc. Natl. Acad. Sci. USA 93, 5507-5511.
15. Muñoz, G., Alcázar, E., Fernández, A., Barragán, C., Carrasco, A., de Pedro, E., Silió, L., Sánchez, J. L. and Rodríguez, M. C. 2010. Effects of porcine MC4R and LEPR polymorphisms, gender and Duroc sire line on economic traits in Duroc × Iberian crossbred pigs. Meat Sci. 88, 169-173.
16. Muñoz, G., Ovilo, C., Silió, L., Tomás, A., Noguera, J. L. and Rodríguez, M. C. 2009. Single- and joint-population analyses of two experimental pig crosses to confirm quantitative trait loci on Sus scrofa chromosome 6 and leptin receptor effects on fatness and growth traits. J. Anim. Sci. 87, 459-468.
17. Ovilo, C., Pérez-Enciso, M., Barragán, C., Clop, A., Rodríguez, C., Oliver, M. A., Toro, M. A. and Noguera, J. L. 2000. A QTL for intramuscular fat and backfat thickness is located on porcine chromosome 6. Mamm. Genome 11, 344-346.
18. Ovilo, C., Oliver, A., Noguera, J. L., Clop, A., Barragán, C., Varona, L., Rodríguez, C., Toro, M., Sanchez, A., Perez-Enciso, M. and Silió, L. 2002. Test for positional candidate genes for body composition on pig chromosome 6. Genet. Sel. Evol. 34, 465-479.
19. Ovilo, C., Clop, A., Noguera, J. L., Oliver, M. A., Barragan, C., Rodriguez, C., Silio, L., Toro, M. A., Coll, A., Folch, J. M., Sanchez, A., Babot, D. and Varona, L. 2002. Quantitative trait locus mapping for meat quality traits in an Iberian xLandrace F2 pig population. J. Anim. Sci. 80, 2801-2808.
20. Ovilo, C., Fernandez, A., Noguera, J. L., Barragan, C., Leton, R., Rodriguez, C., Mercade, A., Alves, E., Folch, J. M., Varona, L. and Toro, M. 2005. Fine mapping of porcine chromosome 6 QTL and LEPR effects on body composition in multiple generations of an Iberian by Landrace intercross. Genet. Res. 85, 57-67.
21. Qian, H., Hausman, G. J., Compton, M. M., Azain, M. J., Hartzell, D. L. and Baile, C. A. 1998. Leptin regulation of peroxisome proliferator-activated receptor-gamma, tumor necrosis factor, and uncoupling protein-2 expression in adipose tissues. Biochem. Biophys. Res. Commun. 246, 660-667.
22. Sampath, H. and Ntambi, J. M. 2005. Polyunsaturated fatty acid regulation of genes of lipid metabolism. Annu. Rev. Nutr. 25, 317-340.
23. Tartaglia, L. A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Richards, G. J., Campfield, L. A., Clark, F. T., Deeds, J., Muir, C., Sanker, S., Mioniarity, A., Moore, K. J., Smutko, J. S., Mays, G. G., Wool, E. A., Monroe, C. A. and Tepper, R. I. 1995. Identification and expression cloning of a leptin receptor, OB-R. Cell 83, 1263-1271.
24. Uemoto, Y., Kikuchi, T., Nakano, H., Sato, S., Shibata, T., Kadowaki, H., Katoh, K., Kobayashi, E. and Suzuki, K. 2012. Effects of porcine leptin receptor gene polymorphisms on backfat thickness, fat area ratios by image analysis, and serum leptin concentrations in a Duroc purebred population. Anim. Sci. J. 83, 375-385.
25. Uleberg, E., Wideroe, I. S., Grindflek, E., Seyda, J., Lien, S. and Meuwissen, T. H. 2005. Fine mapping of a QTL for intramuscular fat on porcine chromosome 6 using combined linkage and linkage disequilibrium mapping. J. Anim. Breed Genet. 122, 1-6.

초록: 독특 바이오티어의 동지방두께와 연관된 kemp evolution유전자의 신규 SNP 마커

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돼지에게 있어서 지방 형질은 가장 중요한 경제형질 중 하나다. 돼지의 렙틴수용체 유전자(LEPR)는 염색체 상의 위치와 그 생리 활성 증상을 통해서 돼지의 렙틴수용체의 총성의 적절한 조절에 영향을 미친다. 본 연구에서는 LEPR 유전자의 구조 변이와 돼지 경제형질과의 연관성을 분석하였다. 이를 위하여 돼지 LEPR 유전자를 포함하고 있는 박테리아 인공 염색체(BAC) 클론에 대한 삽입 염기서열 해독을 수행하여 114 kb 크기의 유전체 서열을 확보하였다. 그리고 전사경로를 코돈으로부터 1.2 kb 상위 영역에서 여러 전사인자 결합부위를 발견하였다. 또한 LEPR 유전자 변이 영역의 6개 SNP와 5개 조절영역의 18개 SNP에 대해 550마우스 두목 개체를 대상으로 연관성 분석을 수행하였다. 이들 SNP 중, -700C/G만이 동지방두께와 경계유 형질과 유의적으로 연관되어 있었으며, 2개의 미스센스 다형성 SNP를 포함하여 다른 SNP에서는 어떤 형질과도 연관성을 보이지 않았다. 결과적으로 -700C/G SNP는 독특 돼지에서 지방과 경계형질을 유전적으로 개량하는데 유용한 마커로 활용될 수 있을 것이다.