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Minimizing a QTL region for intramuscular fat content by characterizing the porcine Phosphodiesterase 4B (*PDE4B*) gene

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Three isoforms of pig *PDE4B* were cloned and classified as two forms: *PDE4B1* and *PDE4B3*, which contain UCR1 and UCR2; and *PDE4B2*, which contains only UCR2. The amino acid sequences of each isoform showed good conservation in human and rat. *PDE4B2* is expressed in a wide range of tissues, but *PDE4B1* and *PDE4B3* are not. Using an informative SNP for the Iberian x Landrace intercross detected from intron 12, a linkage map was constructed. The location of *PDE4B* was estimated at 123.6 cM outside of the QTL-CI (124-128 cM) for IMF. However, the QTL-CI for IMF was reconfirmed with high significance, and its position was narrowed down to an interval of 4 cM (the region defined by markers *PDE4B* and *SW1881*). Using radiation hybrid mapping, *LEPR*, *LEPROT*, *DNAJC6*, *AK3L1* and *AK3L2* were selected as positional and/or functional candidates related to the QTL. [BMB reports 2008; 41(6): 466-471]

INTRODUCTION

Quantitative trait loci (QTL) are genomic locations that affect quantitative trait variations in a population. A major objective of QTL analysis in livestock is to find genetic markers that can be implemented in breeding programs via marker-assisted selection or to develop animal models for human genetic disorders. QTL studies are conducted on several livestock species, including cattle, chicken, and sheep (1-3). According to the Animal QTL Database (http://www.animalgenome.org/QTLdb/), 1675 QTLs, representing 281 traits that are economically important in pork production, have been identified across nearly all chromosomes (4-6). Up to now, 18 QTLs affecting intramuscular fat content (IMF) have been located on *Sus scrofa* chromosome (SSC) 2, 4, 6, 7, and X (7). Eleven of these QTLs are located on SSC6.

Recently, a fine map of the SSC6q QTL region for body composition was constructed using multiple generations of an Iberian × Landrace intercross (8). A QTL for fatness and meat quality traits, including IMF, exists between positions 125 and 132 cM on SSC6, a region defined by the microsatellite markers, *DG32* and *LEPR*. In a different study, a gene-based RH map was constructed with 37 genes and five microsatellites located between *DG32* and *LEPR* (9). Furthermore, QTL analysis was performed using an Iberian × Landrace intercross based on haplotypes of *ACADM*, which is located between *DG32* and *LEPR* (10). The confidence interval (CI) of QTL for IMF was in the range 129-135 cM, and *PDE4B* was inferred to be located within this CI. *PDE4B* belongs to the PDE4 family, which specifically hydrolyzes intracellular cAMP. Different isoforms are produced from this gene by alternative splicing (11). Each isoform differs by a unique block of amino acids at the N-terminal end. Spliced isoforms of *PDE4B* occur in humans and rats (12, 13). However, there are currently no reports regarding *PDE4B* isoforms in pigs. Because *PDE4B* is located close to *LEPR* in the human and mouse genomes, according to the GenBank database, this gene could be a positional candidate for IMF in pigs.

Therefore, we identified porcine *PDE4B* isoforms and polymorphisms for linkage, QTL, and association analyses for fat deposition traits, mainly backfat thickness (BF) and IMF.

RESULTS AND DISCUSSION

Cloning and characterization of porcine *PDE4B* isoforms

Three different isoforms were identified by RT-PCR, RACE-PCR, and sequencing. The *PDE4B1* (GenBank accession no. EU339284) and *PDE4B3* (GenBank accession no. EU189937) long forms contain the upstream conserved region 1 (UCR1) and UCR2. However, the *PDE4B2* (GenBank accession no. EU339285) short form lacks UCR1 and only contains UCR2 (Fig. 1-A). UCR1 and UCR2 are the regulatory domains that control the conformation of the catalytic domain (14). The cAMP-dependent protein kinase (PKA) consensus motif and the extracellular-signal-related protein kinase (ERK) consensus motif (Pro-Xaa-Ser-Pro) are found at the extreme N-terminal

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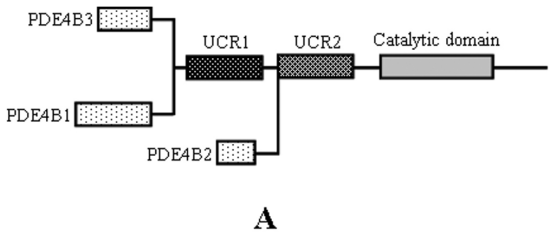

A

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pig   MTAKDSSKELTASESEVCIKTFKEQMHLELELPRLPGNRPTSPKISPRSS  50
Human .............P.....................................
Rat   ....N.....P...S...........R......K.................

pig   PRNSPCFFRKLLVNKSIRQRRRFTVAHTCFDVENGPSPGRSPLDPQASSS 100
Human ..................................................
Rat   ..................................................

                                        UCR1
pig   SGLVLHATFPGHSQRRESFLYRSDSDYDLSPKAMSRNSSLPSEQHGDDLI 150
Human A.....................................................
Rat   S......A..............................................

pig   VTPFAQVLASLRSVRNNFTVLTNLHGTSNKRSPAASQPPVSRVNLPEESY 200
Human ..................I.....................PQ....
Rat   .............I......L.......AP.........A.....SLQ....

                                        UCR2
pig   QKLAMETLEELDWCLDQLETIQTYRSVSEMASNKFKRMLNRELTHLSEMS 250
Human ..................................................
Rat   ..................................................

pig   RSGNQVSEYISNTFLDKQNDVEIPSPTQKDREKKKKQQLMTQISGVKKLM 300
Human ..................................................
Rrat  ..................................................

pig   HSSSLNNTSISRFGVNTENEDHLAKELEDLNKWGLNIFNVAGYSHNRPLT 350
Human ..................................................
Rat   ..................................................

pig   CIMYAIFQERDLLKTFKISSDTLVTYMMTFEDHYHSDVAYHNSLHAADVA 400
Human ................R.....FI.....L.....................
Rat   ................K.....FV.....L.....................

pig   QSTHVLLSTPALDAVFTDLEILAAIFAAAIHDVDHPGVSNQFLINTNSEL 450
Human ..................................................
Rat   ..................................................

pig   ALMYNDESVLENHHLAVGFKLLQEEHCDIFQNLTKKQRQTLRKMVIDMVL 500
Human ...........................M......................
Rat   ...........................Q......................

pig   ATDMSKHMSLLADLKTMVETKKVTSSGVLLLDNYTDRIQVLRNMVHCADL 550
Human ..................................................
Rat   ..................................................

pig   SNPTKSLELYRQWTDRIMEEFFQQGDKERERGMEISPMCDKHTASVEKSQ 600
Human ..................................................
Rat   ..................................................

                                                    ↓
pig   VGFIDYIVHPLWETWADLVQPDAQDILDTLEDNRNWYQSMIPQSPSPPLD 650
Human ..................................................
Rat   ..................................................

pig   EQNRDCQGLMEKFQFELTLEEEDSEGPEKEGEGHGYFGSTKTLCVIDPEN 700
Human ..................D..............S..S.............
Rat   .RS..................E..............PN..S.............

pig   RDSLGETGVDTATEDKSPTDT 721
Human .....DI.I.....V..
Rat   ....E..DI.I.....LI..
```

B

Fig. 1. Structure of pig *PDE4B* isoforms (A) and comparison of the amino acid sequences of *PDE4B3* from three species (B). (A) *PDE4B1* and *PDE4B3* long forms contain the catalytic domain and two upstream conserved regions, UCR1 and UCR2. In contrast, *PDE4B2* short form has the catalytic region and only UCR2. (B) UCR1 and UCR2 are underlined. The catalytic domain is shown in bold letters. Upper and lower arrows indicate the PKA (RRES) and ERK (Pro-Xaa-Ser-Pro) consensus motifs, respectively. The sequence alignment was performed by Clustal W (29).

end of UCR1 and within the catalytic domain, respectively (Fig. 1-B). PKA phosphorylation of a single serine residue increases the activity of the long *PDE4B* isoforms by about 60% (15, 16). In addition, phosphorylation of a serine residue in the ERK motif by ERK reduces its activity (17, 18).

The pig *PDE4B1* and *PDE4B3* isoforms encode proteins of 736 and 721 amino acids, respectively, which are the same lengths as the human and rat genes. However, unlike the human and rat sequences, the porcine *PDE4B2* isoform encodes a protein of 563 amino acids because of the deletion of the 14th amino acid (serine). Thirty-nine amino acids located in the upstream region of UCR2 in pigs showed relatively low identities (89% and 79%) with those of human and rat (data not shown). The amino acid sequences of these three isoforms matched 95% to 97% with the human and rat genes (Fig. 1-B). These high correlations are consistent with previous reports that *PDE4B* isoforms are strongly conserved among several species, including humans and rats (15, 19).

Expression of *PDE4B* isoforms by RT-PCR

The expression patterns of pig *PDE4B* isoforms were verified by RT-PCR using mRNAs isolated from 13 different tissues, including fetal tissue (Fig. 2). Different transcription levels were observed for each isoform. *PDE4B2* was expressed in all tissues, with the strongest intensity in the heart, ovary, small intestine, and lung. *PDE4B1* and *PDE4B3* expression was variable, although they were expressed at similar levels in the fetus. *PDE4B1* was expressed in internal organs, specifically in kidney and lung. However, *PDE4B3* was less expressed in heart and muscle than other tissues. Such differential expression patterns of the spliced isoforms have been reported for several genes in the PDE4 family, suggesting that they have distinct functions (20, 21).

SNP detection from pig *PDE4B*

For SNP identification, the coding regions of each pig *PDE4B* isoform and some intronic regions were targeted for Iberian and Landrace pigs. For the coding region, primers were de-

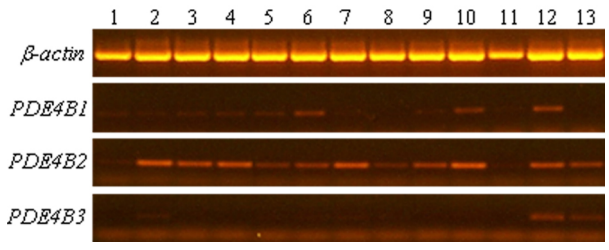

Fig. 2. Tissue distribution of the three pig *PDE4B* isoforms by RT-PCR. Distribution is shown of amplification products separated by electrophoresis on a 2% agarose gel for primer sets specific for *PDE4B1*, *PDE4B2*, and *PDE4B3*. *β-actin* was used as a positive control. Lanes are: 1, brain; 2, heart; 3, liver; 4, ovary; 5, testis; 6, kidney; 7, small intestine; 8, navel; 9, spleen; 10, lung; 11, skin; 12, fetus; and 13, muscle.

signed using determined mRNA sequences. With PCR amplification and direct sequencing, no SNPs were detected in the coding regions of *PDE4B* isoforms. In the next phase, we searched for SNPs in intronic regions, specifically introns 11, 12, and 13. Ultimately, a unique SNP between Iberian and Landrace pigs was detected in intron 12 (data not shown).

Linkage mapping, QTL analysis, and the association study

A SSC6 linkage map was constructed using the SNP in *PDE4B*, 15 microsatellites, and SNPs in the *MC1R*, *ACADM*, and *LEPR* with CRIMAP software version 2.4 (Fig. 3). The sex-averaged map was as follows: *MC1R* - (12.5) - *S0035* - (14.8) - *Sw1329* - (26.5) - *Sw1057* - (12.3) - *S0087* - (15.8) - *Sw1376* - (10.2) - *Sw316* - (7.8) - *Sw71* - (8.8) - *S0228* - (3.0) - *DG32* - (2.4) - *ACADM* - (1.9) - *S0121* - (3.1) - *PDE4B* - (1.3) - *LEPR* - (3.4) - *SW1881* - (3.4) - *DG93* - (26.8) - *SW1324* - (7.3) - *Sw2419* - (3.9) - *Sw607* ($\log_{10}$_likelihood = −2199.32). The total length was 170.3 cM, and the mean distance between markers was 9.4 cM. *PDE4B* was located at 123.6 cM, which placed it between *ACADM* (118.6 cM) and *LEPR* (124.9 cM).

The QTL analysis and association study were performed using the intronic SNP in *PDE4B*. QTLs for all traits used were detected with high LR scores (Table 1). In addition, confidence intervals of QTLs for BF1, BF3, and IMF overlapped in a one cM region (124-125 cM). The confidence interval of the QTL for IMF was narrow at an interval of 4 cM (124-128 cM). However, *PDE4B* was located at 123.6 cM, outside of the QTL-CI for IMF. Moreover, in a marker-assisted association test, there were no statistically significant associations with the three different BF traits and IMF (data not shown).

Chromosomal localization by RH map

An RH map based on QTL for IMF was previously constructed with 37 genes, excluding *PDE4B* (9). Primer sets were designed for *PDE4B* using sequences analyzed in this study, and *PDE4B* and two microsatellite markers, *S0121* and *SW1881*, were typed using the IMNpRH2$_{12,000rad}$ panel (22). With the scoring results of these three markers and 15 genes previously scored, a new RH map was constructed using the RHMAXLINK program (23) (Fig. 3).

In single QTL analyses, the QTL-CI for IMF was detected at 124-128 cM. *LEPR* was located at 124.9 cM and was contained within the QTL-CI for IMF. However, *SW1881* was located outside of QTL-CI. Moreover, according to the RH map, only *LEPR*, *DNAJC6*, and *AK3L1* were between *PDE4B* and *SW1881*. Although the closest gene to *AK3L1* is Janus kinase 1 in the NCBI human genome view build 36.1, the distance between these two genes is about 0.4 Mb. These results suggest that *LEPR*, *DNAJC6*, and *AK3L1* are located within the QTL-CI for IMF. Human and mouse genomic sequences of *LEPROT* and *AK3L2* are contained within those of *LEPR* and *AK3L1*, respectively. Finally, based on their genomic position, *LEPR*,

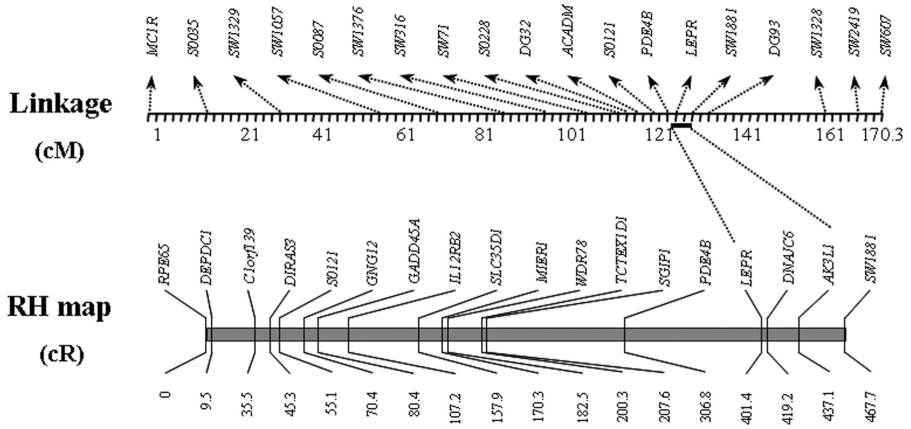

Fig. 3. Comparison of the linkage map with the RH map based on QTL for IMF. The linkage map was established based on a SNP identified in intron 12 of pig *PDE4B* using CRIMAP software version 2.4. *PDE4B* is located at 123.6 cM. The bold bar in the linkage map indicates the QTL region for IMF (124-128 cM). In the RH map, constructed by the RHMAXLINK program, QTL-CI contains three genes, *LEPR*, *DNAJC6*, and AK3L1.

Table 1. QTL detection on SSC6 by single QTL analysis

Trait	LR	P	Position (CI)[a]	a	SE	d	SE
BF1	58.0	2×10^{-11}	125 (119-127)	0.31	0.04	−0.07	0.05
BF2	70.5	4×10^{-14}	109 (106-113)	0.27	0.03	−0.09	0.04
BF34	83.1	0.0	122 (120-125)	4.35	0.47	−2.14	0.65
IMF	53.7	2×10^{-10}	126 (124-128)	0.25	0.03	−0.17	0.05

[a] Position is given in cM, with confidence intervals in parentheses.
LR, likelihood ratio test; P, nominal P value; a, additive effect; SE, standard error; d, dominance effect.

LEPROT, *DNAJC6*, *AK3L1*, and *AK3L2* are possible positional candidates related to IMF in pig.

MATERIALS AND METHODS

Amplification and sequencing of pig *PDE4B*

Total RNA was separately isolated from three tissues (liver, spleen, and muscle) of the Iberian pig and five tissues (brain, heart, liver, ovary, and muscle) of the Landrace pig using Trizol (Gibco BRL, USA). First-strand cDNA synthesis was performed using the Smart™ RACE cDNA Amplification Kit (Clontech, USA). Three expressed sequence tags (EST) sequences were obtained (GenBank accession no. BX925195, BM083204, and BI400362) from BLAST searches using the human *PDE4B* sequence (GenBank accession no. NM_002600) in the TGI database (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/Blast/index.cgi). Two primer sets were designed using these three EST sequences for the amplification of a nearly full coding sequence (CDS) as follows: 1F, 5'-ACAGCCAGCGCCGAGAATCC-3'; 1R, 5'-GGTAACAAGCGTGTCCGAGG-3'; 2F, 5'-CCTCGGACACGCTTGTTACC-3'; 2R, 5'-GCCAGCAAGGCAAGTCAACC-3'. All PCR reactions were performed on a PTC-200 programmable thermal controller (MJ Research, Inc., USA). The PCR was carried out in a total volume of 25 μL containing 25 ng of cDNA and 1.5 units of *Taq* polymerase (Takara, Japan). A 2 min denaturation step at 94°C was followed by 35 cycles (45 s at 94°C, 60 s at 62°C, and 90 s at 72°C) and a final extension of 5 min at 72°C. The PCR products were cloned using the TOPO TA Cloning Kit (Invitrogen, USA). All sequencing was performed on an Applied Biosystems 3700 DNA sequencer (PE Applied Biosystems, USA).

Rapid amplification of cDNA ends (RACE)

To obtain the full-length CDS, specific primers were designed as follows: first (5'-ATCGCCGTGTTGCTCGCTGG-3') and nested (5'-CATAGTCGCTGTCTGATCGG-3') primers for the 5'-UTR; and first (5'-CCAGAGTGCAAGACCGTGAAGC-3') and nested (5'-GGTTGACTTGCCTTGCTGGC-3') primers for the 3'-UTR. First and nested PCRs were performed in a total volume of 50 μL using the Smart™ RACE cDNA Amplification Kit (Clontech, USA), according to the manufacturer's protocol. The RACE-PCR products were cloned and sequenced. For discovery of pig *PDE4B* 5' variants, primers for each isoform were designed based on the known 5' variants from human and rat. For PDE4B1, PDE4B3, and PDE4B4, first (5'-ATCGCCGTGTTGCTCGCTGG-3') and nested (5'-CATAGTCGCTGTCTGATCGG-3') primers were used, and for PDE4B2, first (5'-GGAAGCCATCTCACTGACAGAGC-3') and nested (5'-GGTCTGGATGGTCTCTAGCTGGAC-3') primers were used. RACE-PCRs were performed with the same procedure used for previous CDS analysis, and the RACE-PCR products were cloned and sequenced.

RT-PCR for mRNA expression of each isoform

RT-PCR was performed to verify the expression patterns of pig *PDE4B* isoforms (24, 25). Total RNA was extracted from 13 tissues from Landrace pig using Trizol (Gibco BRL, USA), and mRNAs were purified using the PolyATtract mRNA Isolation System (Promega, USA), according to the manufacturer's protocol. First-strand cDNA synthesis was performed using the Smart™ RACE cDNA Amplification Kit (Clontech, USA). A primer set used for amplification of each isoform was designed using the 5'-UTR sequences or CDS as follows: PDE4B1-F, 5'-GACGATTTCGAATGTAGCTTGAG-3'; PDE4B1-R, 5'-GCAATGCTTGGAAGCGTCATC-3; PDE4B2-F, 5'-GTGATTTGCTCTCCTGGTGG-3'; PDE4B2-R, 5'-CATGTAGTTAGGTTGGAGCG-3'; PDE4B3-F, 5'-GTTCTTGCTCTAAGCAGCTCC-3'; and PDE4B3-R, 5'-GGTGCTGGCTTTCACCATCC-3'. PCR was performed in a total volume of 25 μL containing 50 ng of cDNA and 2 units of *Taq* polymerase (Takara, Japan). A 2 min denaturation step at 94°C was followed by 35 cycles (30 s at 94°C, 60 s at 66°C for PDE4B1, 60°C for PDE4B2, or 63°C for PDE4B3, and 90 s at 72°C) and a final extension of 5 min at 72°C. The PCR products were separated on 2% agarose gels and visualized with ethidium bromide. *β-actin* levels were used as internal standards for the determination of targeted mRNA levels.

Mutation detection in Iberian and Landrace pigs

Primers were designed to amplify the coding region and relatively short intronic regions of introns 11, 12, and 13 as follows: B1-F, 5'-TTGTGCCGGAGGAGTACTGC-3'; B1-R, 5'-CTACCATACAGCTGGCATGC-3'; B2-F, 5'-GTGATTTGCTCTCCTGGTGG-3'; B2-R, 5'-CTACCATACAGCTGGCATGC-3'; B3-F, 5'-GTTCTTGCTCTAAGCAGCTCC-3'; B3-R, 5'-CTACCATACAGCTGGCATGC-3'; in11-F, 5'-AGGAGCTGGAAGACCTGAAC-3'; in11-R, 5'-GAACATGGGTTGACTGGGCC-3'; in12-F, 5'-AAATCTCCTCGGACACGCTTG-3'; in12-R, 5'-AACTGATTGGAGACTCCAGG-3'; in13-F, 5'-TCCAGCAGGGAGACAAAGAG-3'; and in13-R, 5'-GAGGATGGACAATGTAGTCG-3'. For the coding region, three forward primers were designed using the unique sequences of the N-terminal regions of each isoform and a reverse primer using the identical 3'-UTR sequence. cDNA for the coding region and genomic DNA for the intronic regions were used as the templates for amplification. The amplifications were performed in a total volume of 25 μL containing 25 ng genomic DNA or 50 ng cDNA and 1.5 unit of *Taq* polymerase (Takara, Japan). A 2 min denaturation step at 94°C was followed by 35 cycles (30 s at 94°C, 45 s at 53°C for coding region or 62°C for intronic region, and 90 s at 72°C) and a final extension of 5 min at 72°C. The PCR products were prepared using GELase™ Agarose Gel-Digesting Preparation (Epicentre, USA) and then directly sequenced. The sequences determined were aligned and analyzed for SNP detection using Sequencher ver. 4.6 (Gene codes, USA).

Restriction fragment length polymorphism (RFLP) for genotyping

A SNP identified from intron 12 was genotyped by PCR-RFLP in the Iberian × Landrace resource population (33 F_0, 70 F_1, 419 F_2, 86 F_3, and 126 backcross individuals). A primer set (5'-AAATCTCCTCGGACACGCTTG-3' and 5'-TTCTAGGGCC-ACTTCCTTCG-3') was designed for amplification. PCR was performed in a total volume of 15 μL containing 15 ng of genomic DNA and 1 unit of *Taq* polymerase (Takara, Japan). A 2 min denaturation step at 94°C was followed by 35 cycles (30 s at 94°C, 30 s at 62°C, and 30 s at 72°C) and a final extension of 5 min at 72°C. The *Hin*fI enzyme (New England Biolabs, UK) was used to digest the PCR products. Digested DNA was separated on a 4% agarose gel and stained with ethidium bromide.

Statistical analysis

Phenotypic traits analyzed in the present work were backfat at three different locations (BF1, BF2, and BF34) and intramuscular fat percentage (IMF) (8). Linkage mapping was performed using the build option of the CRIMAP software version 2.4 (26). Two models were used to analyze the data, (A) a QTL model and (B) an association model to perform the marked-assisted association test (27).

$$y_i = \text{sex}_i + \text{batch}_i + \beta_c\, ci + c_a\, a + c_d\, d + u_i + e_i\,, \quad \text{(A)}$$

$$y_i = \text{sex}_i + \text{batch}_i + \beta_c\, ci + \lambda_i\, g + u_i + e_i\,, \quad \text{(B)}$$

where y_i is the i-th individual record, batch is the slaughter batch, β_c is a covariate coefficient with c being carcass weight, a is the QTL additive effect, d is the dominant effect, and c_a and c_d are the additive and dominant coefficients, respectively. In (B), λ is a $-1/1$ indicator variable depending on whether the individual is homozygous for the alternative alleles of a SNP (heterozygous animals were given a value of 0), and g represents the allele effect. Finally, u is the infinitesimal genetic effect and e is the residual. All statistical analyses were carried out with Qxpak (28).

Construction of radiation hybrid map

Primers for amplification of *PDE4B* were designed using 3'-UTR sequences as follows: RH-F, 5'-GGCTCAGGAATCC-CTCGGT-3' and RH-R, 5'-AGACAAGGGGACAGGTGAGC-3'. In addition, primers for *S0121* and *SW1881* were designed using known sequences (GenBank accession no. L30152 and AF253728) as follows: S0121-F, 5'-TTGTACAATCCCAGTGG-AATCC-3'; S0121-R, 5'-AATAGGGCATGAGGGTGTTTGA-3'; SW1881-F, 5'-TGACCCAGCAAGTCTTCTGG-3'; and SW1881-R, 5'-CGGAAATACATTTTTATGGCTG-3'. An IMNpRH2$_{12,000rad}$ panel was used in this study. Each PCR contained 25 ng of hybrid DNA and 0.3 units of *Taq* polymerase (Genetbio, Korea) in a total volume of 15 μL. The mixture was treated at 94°C for 2 min, followed by 35 cycles (30 s at 94°C, 30 s at the appropriate annealing temperature, and 30 s at 72°C), and a final extension of 5 min at 72°C. Annealing temperatures for *PDE4B*, *S0121*, and *SW1881* were 64°C, 65°C, and 61°C, respectively. After scoring by electrophoresis, a RH map was constructed using the RHMAP software package (version 3.0) (23).

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REFERENCES

1. Abasht, B., Dekkers, J. C. M. and Lamont, S. J. (2006) Review of Quantitative trait loci identified in the chicken. *Poultry Sci.* **85,** 2079-2096.
2. Karamichou, E., Richardson, R. I., Nute, G. R., Gibson, K. P. and Bishop, S. C. (2006) Genetic analyses and quantitative trait loci detection, using a partial genome scan, for intramuscular fatty acid composition in Scottish Blackface sheep. *J. Anim. Sci.* **84,** 3228-3238.
3. Khatkar, M. S., Thomson, P. C., Tammen, I. and Paadsma, H. W. (2004) Quantitative trait loci mapping in dairy cattle: review and meta-analysis. *Genet. Sel. Evol.* **36,** 163-190.
4. Bidanel, J. P. and Rothschild, M. F. (2002) Current status of quantitative trait locus mapping in pigs. *Pig News Info.* **23,** 39N-53N.
5. Nagamine, Y., Haley, C. S., Sewalem, A. and Visscher, P. M. (2003) Quantitative trait loci variation for growth and obesity between and within lines of pigs (*Sus scrofa*). *Genetics* **164,** 629-635.
6. Rohrer, G. A., Thallman, R. M., Shackelford, S., Wheler, T. and Koohmaraie, M. (2006) A genome scan for loci affecting pork quality in a Duroc-Landrace F population. *Anim. Genet.* **37,** 17-27.
7. Rothschild, M. F., Hu, Z. I. and Jiang, Z. (2007) Advances in QTL mapping in pigs. *Int. J. Biol. Sci.* **3,** 192-197.
8. 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.
9. Kim, J. H., Lim, H. T., Park, E. W., Ovilo, C., Lee, J. H. and Jeon, J. T. (2006) A gene-based radiation hybrid map of the pig chromosome 6q32 region associated with a QTL for fat deposition traits. *Anim. Genet.* **37,** 522-523.
10. Kim, J, H., Lim, H. T., Park, E. W., Rodríguez, C., Silio, L., Varona, L., Mercade, A., Jeon, J. T. and Ovilo, C. (2006) Polymorphisms in the promoter region of the porcine *acyl-coA dehydrogenase, medium-chain (ACADM)* gene have no effect on fat deposition traits in a pig Iberian × Landrace cross. *Anim. Genet.* **37,** 430-431.
11. Houslay, M. D., Sullivan, M. and Bolger, G. B. (1998) The multienzyme PDE4 cyclic adenosine monophosphate-

specific phosphodiesterase family; Intracellular targeting, regulation, and selective inhibition by compounds exerting anti-inflammatory and antidepressant actions. *Adv. Pharmacol.* **44,** 225-342.
12. Huston, E., Lumb, S., Russell, A., Catterall, C., Ross, A. H., Steele, M. R., Bolger, G. B., Perry, M. J., Owens, R. J. and Houslay, D. (1997) Molecular cloning and transient expression in COS7 cells of a novel human PDE4B cAMP-specific phosphodiesterase, HSPDE4B3. *Biochem. J.* **328,** 549-558.
13. Shepherd, M., McSorley, T., Olsen, A. E., Johnston, L A., Thomson, N. C., Baillie, G. S., Houslay, M. D. and Bolger, G. B. (2003) Molecular cloning and subcellular distribution of the novel *PDE4B* cAMP-specific phosphodiesterase isoform. *Biochem. J.* **370,** 429-438.
14. Conti, M., Richter, W., Mehats, C., Livera, G., Park, J. Y. and Jin, C. (2003) Cyclic AMP-specific PDE4 phosphodiesterases as critical components of cyclic AMP signals. *J. Biol. Chem.* **278,** 5493-5496.
15. Hoffmann, R., Wilkinson, I. R., McCallum, J. F., Engels, P. and Houslay, M. D. (1998) cAMP-specific phosphodiesterase HSPDE4D3 mutants which mimic activation and changes in rolipram inhibition triggered by protein kinase A phosphorylation of Ser-54: generation of a molecular model. *Biochem. J.* **333,** 139-149.
16. Sette, C. and Conti, M. (1996) Phosphorylation and activation of a cAMP-specific phosphoesterase by the cAMP-dependent protein kinase. Involvement of serine 54 in the enzyme activation. *J. Biol. Chem.* **274,** 16526-16534.
17. Baillie, G, S., MacKenzie, S, J., McPhee, I. and Houslay, M. D. (2000) Sub-family selective actions in the ability of Erk2 MAP kinase to phosphorylate and regulate the activity of PDE4 cAMP-specific phosphoesterases. *Br. J. Pharmacol.* **131,** 811-819.
18. Hoffmann, R., Baillie, G. S., Mackenzie, S. J., Yarwood, S. J. and Houslay, M. D. (1999) The MAP kinase ERK2 inhibits the cAMP-specific phosphoesterase, HSPDE4D3 by phosphorylating it at Ser579. *EMBO J.* **18,** 893-903.
19. Bolger, G. B., Rodgers, L. and Riggs, M. (1994) Differential CNS expression of alternative mRNA isoforms of the mammalian genes encoding cAMP-specific phosphodiesterase. *Gene* **149,** 237-244.
20. Iona, S., Cuomo, M., Bushnik, T., Naro, F., Sette, C., Hess, M., Shelton, E. R. and Conti, M. (1998) Characterization of the rolipram-sensitive, cyclic AMP-specific phosphodiesterases: identification and differential expression of immunologically distinct forms in the rat brain. *Mol. Pharmacol.* **53,** 23-32.
21. McPhee, I., Cochran, S. and Houslay, M. D. (2001) The novel long PDE4A10 cyclic AMP phosphodiesterase shows a pattern of expression with brain that is distinct from the long PDE4A5 and short PDE4A1 isoforms. *Cell Signalling* **13,** 911-918.
22. Yerle, M., Pinton, P., Delcros, C., Arnal, N., Milan, D. and Robic, A. (2002) Generation and characterization of a 12,000-rad radiation hybrid panel for fine mapping in pig. *Cytogenet. Genome Res.* **97,** 219-228.
23. Boehnke, M., Lange, K. and Cox, D. R. (1991) Statistical methods for multipoint radiation hybrid mapping. *Am. J. Hum. Genet.* **49,** 1174-1188.
24. Zhong, J., Wang, Y., Qiu, X., Mo, X., Liu, Y., Li, T., Song, Q., Ma, D. and Han, W. (2006) Characterization and expression profile of CMTM3/CKLFSF3. *J. Biochem. Mol. Biol.* **39,** 537-545.
25. Tang, W., Yuan, J., Chen, X., Gu, X., Luo, K., Li, J., Wan, B., Wang, Y. and Yu, L. (2006) Identification of a novel human lysophosphatidic acid acyltransferase, LPAAT-theta, which activates mTOR pathway. *J. Biochem. Mol. Biol.* **39,** 626-635.
26. Green, P., Falls, K. and Crooks, S. (1990) Documentation of CRIMAP. Unpublished mimeo (http://Biobase.Embnet-tut/crimap).
27. Zhao, H., Rothschild, M. F., Fernando, R. L. and Dekkers, J. C. M. (2003) Tests of candidate genes in breed cross populations for QTL mapping in livestock. *Mamm. Genome* **14,** 472-482.
28. Pérez-Enciso, M. and Mizztal, I. (2004) Qxpak: a versatile mixed model application for genetical genomics and QTL analyses. *Bioinformatics* **20,** 2792-2798.
29. Thompson, J. D., Higgins, D. G. and Gibson, T. J. (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positive-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22,** 4673-4680.