GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Differential expression and effect of the porcine *ANGPTL4* gene on intramuscular fat

Z.Q. Ren[1]*, W.J. Wu[1,3]*, W.H. Liu[1,2], R. Zheng[1], J.L. Li[1], B. Zuo[1], D.Q. Xu[1], F.E. Li[1], M.G. Lei[1], D.B. Ni[1,2] and Y.Z. Xiong[1]

[1]Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture & Key Laboratory of Agriculture Animal Genetics, Breeding and Reproduction, Ministry of Education, College of Animal Science, Huazhong Agricultural University, Wuhan, China
[2]China Swine Testing Center, College of Animal Science, Huazhong Agricultural University, Wuhan, China
[3]College of Animal Science, Nanjing Agricultural University, Nanjing, China

*These authors contributed equally to this study.
Corresponding author: Z.Q. Ren
E-mail: renzhuqing@163.com

Genet. Mol. Res. 13 (2): 2949-2958 (2014)
Received February 4, 2013
Accepted July 17, 2013
Published April 16, 2014
DOI http://dx.doi.org/10.4238/2014.April.16.3

ABSTRACT. In a previous study, we investigated differences in gene expression in backfat between Meishan and Large White pigs and their F1 hybrids, Large White x Meishan, and Meishan x Large White pigs. One potential differentially expressed sequence tag from the mRNA differential display was a homolog of the human *angiopoietin-like 4* (*ANGPTL4*) gene, which encodes a protein that is secreted by both liver and white adipose tissues and can inhibit lipoprotein lipase activity and stimulate white adipose tissue lipolysis. Here, *ANGPTL4* mRNA was found to be upregulated in the backfat of Large White compared with that in the Meishan pigs and the F1 hybrids, Meishan x Large White and Large White x Meishan, whereas expression was lowest both in the longissimus dorsi and the heart, as shown by the tissue distribution profile. Only one mutation, a G/A transition located in the third intron, was found. The

ANGPTL4 G/A polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) showed a significant effect on intramuscular fat (IMF), water moisture of the longissimus dorsi, meat marbling of the longissimus dorsi, and pH of the longissimus dorsi ($P < 0.05$). This site seemed to be significantly ($P < 0.05$) additive in its actions on IMF, water moisture, and pH, whereas it showed significant dominance in its action on meat marbling ($P < 0.05$). This locus can be potentially considered as a marker for IMF improvement.

Key words: *ANGPTL4*; Differential expression; Association analysis; Intramuscular fat; Pigs

INTRODUCTION

In the past several decades, much progress has been achieved in porcine breeding, such as the improvement of lean meat percentage of the carcass, and growth rate among others. However, the intramuscular fat (IMF) content, or marbling, which largely affects meat quality, has been relatively ignored so far. As the focus shifts away from the above traits, the opportunity to breed for meat quality, and specifically to improve IMF quality, should not be missed. IMF is the quality of a pig that enhances its taste, tenderness, and palatability (Hovernier et al., 1993; Hocquette et al., 2010).

The basis of the accumulation of IMF is so complicated that it is highly associated with nutrition, genetic background, and development of livestock. From a nutritional perspective, the deposition of IMF has a strong impact on nutritional and technological properties of swine meat (Wood et al., 2008). It is well known that the development of adipocytes is fixed relatively early in life and that subsequent processing affecting both size and number occurs in proportion to the initial cell number and lipogenic proteins (Caserta et al., 2001; Pethick et al., 2004). Therefore, to improve IMF, the first and foremost approach is to determine a genetically based method for accumulating more adipocytes in muscle.

In a previous study, we used an mRNA differential display technique to analyze differential gene expression in backfat between Meishan and Large White pigs and their F1 hybrids, Large White x Meishan and Meishan x Large White pigs (Ren et al., 2005). Many expressed sequence tags were isolated and identified. One potential differentially expressed sequence tag, designated as F55, was homologous to human angiopoietin-like 4 (ANGPTL4).

ANGPTL4, one of the members of the angiopoietin-like protein family, is a secreted protein that can inhibit lipoprotein lipase (LPL) activity via the conversion of LPL dimers to monomers (Sukonina et al., 2006), and stimulate white adipose tissue lipolysis. ANGPTL4 was considered as a novel signal involved in the regulation of lipid and glucose metabolism, especially under fasting conditions (Kersten et al., 2000; Oike et al., 2005; Mandard et al., 2006). In pigs, very little is known about the angiopoietin-like gene. In the present study, we cloned the porcine *ANGPTL4* gene, determined its differential expression patterns between Meishan and Large White pigs and their F1 hybrids of both direct and reciprocal crosses, and evaluated the effect of a G/A transition of *ANGPTL4* on IMF and other meat quality traits in a population derived from crossing Chinese Meishan and Large White pigs with the aim to identify DNA markers that could be used for association studies on fat traits in pigs.

MATERIAL AND METHODS

Animals

In the present study, the F2 generation (86 dams and 94 sires) of an intercross between British Large White boars and Chinese Meishan sows was used for association analysis. All pigs were fed the same diets, which were formulated according to age under a standardized feeding regimen, and had free access to water at the Jingpin Pig Station of Huazhong Agricultural University. Pigs were slaughtered at the age of 6 months, and subsequently, the porcine traits' phenotypic values were measured, including pH of them, longissimus dorsi, pH of the biceps femoris muscle, IMF of the longissimus dorsi, water holding capacity, water moisture of the longissimus dorsi, meat marbling of the longissimus dorsi, meat marbling of the biceps femoris, and meat color value of the longissimus dorsi. Genomic DNA was isolated from blood samples using a standard phenol:chloroform extraction method.

Reverse transcription (RT) and differential display RT-PCR (polymerase chain reaction) of mRNA

Total RNA isolated using TRIzol Reagent (Invitrogen, USA) from porcine backfat at the thorax-waist was treated with RNase-free DnaseI (Promega, USA) to remove potential contaminating genomic DNA. M-MLV reverse transcriptase and Oligo dT15 anchored primers (Promega) were used to synthesize cDNA.

Differential display PCR and non-denaturing polyacrylamide gel electrophoresis were employed as described by Ren et al. (2005). The cDNA fragments differentially displayed on gel were re-amplified using the differential display primer set (Anchor3 and Arbitrary5; Table 1), then cloned and sequenced.

Table 1. Primers used in the present study.

Primers	Sequences (5' to 3')	Annealing temperature (°C)	Length of amplified fragment (bp)	Mg^{2+} (mM)
Anchor3	ACGACTCACTATAGGGCTTTTTTTTTTTGT			
Arbitrary5	ACAATTTCACACAGGAATGGTCGTCT			
GHF	ACCACAAGTCCATGCCATCAC	58	480	1.5
GHR	TCCACCACCCTGTTGCTGTA			
SF55F	GCCTTGGGAAGCCTACAA	54	164	1.5
SF55R	CCAGGTGGACGGGGAACT			
SMART5'	AACGCAGAGTACGCGGG	53	1602	1.5
GSP2	TGTGCCTCCGTGCCTCGC			
SMART3'	CAGAGTACTTTTTTTTTTTTTTTT	52	473	2.5
GSP1	TGGCCCAGCATGTGGCCA			
RGHF	GTGCTGAGTATGTCGTGGAGT	60	288	1.5
RGHR	TCTTCTGGGTGGCAGTGAT			
RPARF	GGAGAAGCAGCACTTGAGAA	60	146	1.5
RPARR	GGGTCATCTTGGGTAGTCTTT			
AL1F	GCTGGAACGGCGTCTGA	54	383	2.0
AL1R	TGGATTCTCAAGTGCTGCTT			
AL2F	GGAGAAGCAGCACTTGAGAA	58	245	2.0
AL2R	GGGTCATCTTGGGTAGTCTTT			
AL3F	GCCAAGGCTGCCAGGAGA	57	3530	2.0
AL3R	ACCAGGAATGGCGGGGAC			
AL4F	AGAGCGGCAAAGTGGATTG	59	441	2.0
AL4R	GCGCCTCTGAATTACAGTCC			
AL5F	GATGGCTCGGTGGACTTT	55	422	2.0
AL5R	TTGGCGCAGTTCTTGTCT			
AL6F	CAAGACTGGGAGGGCAATG	56	1793	2.0
AL6R	CGAGGGATGGAATGGAAGT			

Semi-quantitative RT-PCR analysis of F55

Six pigs comprised of three boars and three sows from each Meishan x Large White, Large White x Meishan, Meishan, and Large White population, that is 24 pigs in total, were sampled for total RNA isolation. Semi-quantitative RT-PCR was used to identify *ANGPTL4* expression in backfat of Meishan and Large White pigs and their F1 hybrids. A specific primer pair, GHF and GHR (Table 1), was synthesized to amplify the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as the control. The differential display cDNA F55 fragment was detected with the gene-specific primers SF55F and SF55R (Table 1). *GAPDH* and F55 were amplified separately and electrophoresed on 1.5% agarose gels with ethidium bromide. Densitometry values were measured using the BandScan software (www.Glyko.com). RT-PCR values are presented as the ratio of the F55 signal in the selected exponential amplification cycle divided by the *GAPDH* signal. Each sample was repeated eight times in duplicate PCRs.

Cloning of *ANGPTL4* cDNA and genomic DNA

Switching Mechanism At the 5'-end of the RNA Transcript (SMART) cDNA was synthesized using the SMART PCR cDNA Synthesis Kit (Clontech, USA) for rapid amplification of cDNA ends (RACE)-PCR. The 5'- and 3'-ends of *ANGPTL4* cDNA were obtained using the primer pairs Smart5'/GSP1 and Smart3'/GSP2, respectively (Table 1).

The genomic sequence of *ANGPTL4* was also amplified using six primer pairs (AL1F and AL1R, AL2F and AL2R, AL3F and AL3R, AL4F and AL4R, AL5F and AL5R, and AL6F and AL6R; Table 1), which were designed based on the exon/intron boundaries of human *ANGPTL4*.

Real-time RT-PCR analysis

Relative quantitative RT-PCR was performed using an MJ Option2 detection system (MJ Research, USA) with the following protocol: denaturation at 95°C for 2 min; amplification by 45 cycles each at 95°C for 30 s and annealing and extension at 60°C for 30 s and at 72°C for 18 s, respectively. The sequences of *GAPDH*- and *ANGPTL4*-specific primers are presented in Table 1 (RGHF and RGHR for *GAPDH*; RPARF and RPARR for *ANGPTL4*).

For spatial expression analysis, total RNAs were isolated from various tissues, including backfat, longissimus dorsi, heart, liver, spleen, lung, kidney, stomach, uterus, ovary, and small intestine of Meishan pigs. Each sample was repeated four times and the comparative cycle threshold (C_t) ($^{\Delta\Delta}C_t$) value method (Livak and Schmittgen, 2001) was used to compute relative quantification. Expression levels were considered undetectable when the C_t value of the targeted gene exceeded 35 in the tissue sample.

Association analysis of porcine *ANGPTL4*

Association analyses were performed in our experimental populations containing 259 Meishan x Large White F2 pigs. We utilized a general linear model program of the SAS version 8.1 software package (SAS Institute, USA) to evaluate the associations between genotypes and fat traits. Both additive and dominant effects were estimated using REG procedures

of SAS version 8.1, where the additive effect was denoted as -1, 0, and 1 for genotypes AA, AB, and BB, respectively, and the dominance effect was represented as 1, -1, and 1 for genotypes AA, AB, and BB, respectively. The model of the SAS program was as follows:

$$Y_{ijklm} = \mu + G_i + S_j + B_k + W_l + A_m + e_{ijklm}$$

where μ is the population mean, Y_{ijklm} is the phenotypic value of the target trait, G_i is the genotype effect, S_j is the sex effect, B_k is the boar effect, W_l is the regression coefficient of the slaughter weight, A_m is the regression coefficient of the slaughter age, and e_{ijklm} is the random error effect for each observation.

RESULTS

Identification of downregulated expression of F55 in Large White x Meishan crossbred pigs

A band designated as F55 that was observed at low expression levels in Large White x Meishan crossbred pigs was isolated from the differential display gel (Figure 1A) and re-amplified. Semi-quantitative RT-PCR analysis revealed that ESTS30 presented lower expression in the backfat of Large White x Meishan crossbred pigs relative to that of their parents and Meishan x Large White F1 pigs (Figure 1B and C).

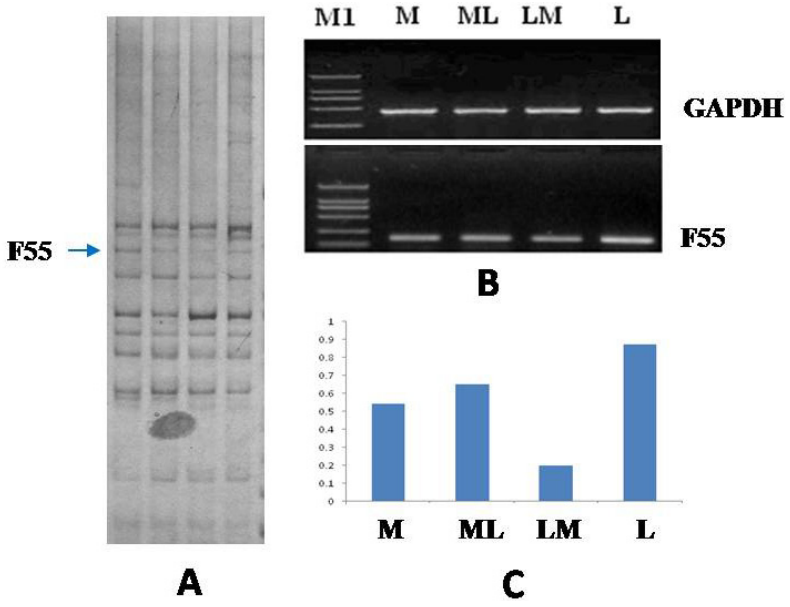

Figure 1. Identification of F55 as differential expression in backfat of Large White and Meishan pigs and their F1 hybrids. **A.** Silver staining of differential display. The arrow points to F55. M, ML, LM and L represent Meishan, Meishan x Large White, Large White x Meishan and Large White pigs, respectively. M1 is the DNA marker with the size of 2000, 1000, 750, 500, 250, and 100 bp. **B.** Semi-quantitative RT-PCR analysis of ESTS30. **C.** Bar graph of the percentage of F55/*GAPDH*.

Cloning cDNA and genomic organization of porcine *ANGPTL4*

Using 5'- and 3'-RACE-PCR, we obtained the 1847-bp full-length porcine *ANGPTL4* cDNA (Figure 2).

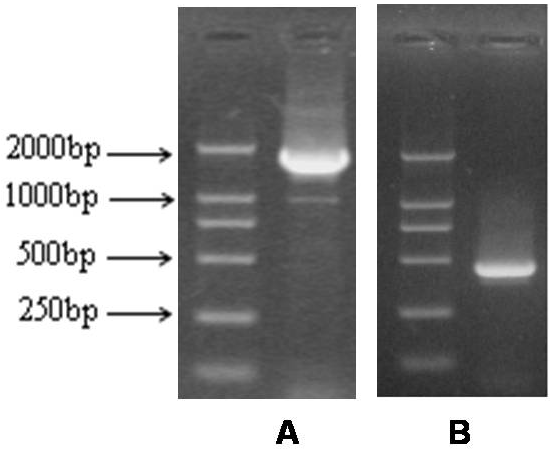

Figure 2. RACE-PCR amplification of 5'-end (**A**) and 3'-end (**B**) of porcine *ANGPTL4*.

Porcine *ANGPTL4* contained an open reading frame of 1239 nucleotides encoding a protein of 412 amino acids.

We also obtained a 7394-bp genomic DNA sequence covering the full length of the coding sequence of porcine *ANGPTL4* and elucidated the exon/intron boundary (Figure 3). The genomic structure of *ANGPTL4* is organized in seven exons, separated by six introns. The locations of splice donor/acceptor sites in all introns conformed to the consensus GT/AG rule (Breathnach et al., 1978).

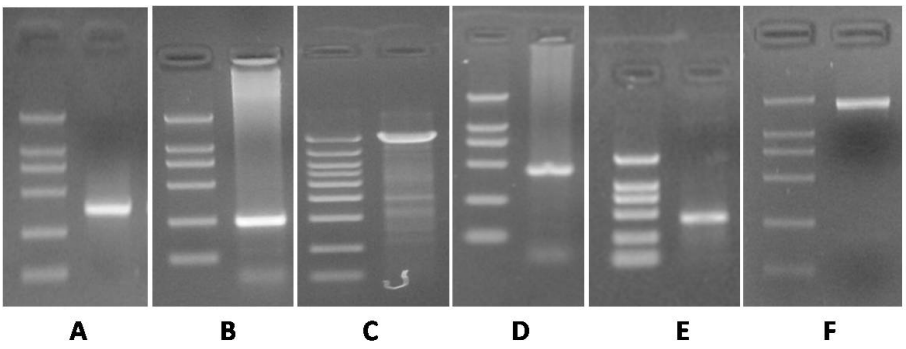

Figure 3. Amplification of porcine *ANGPTL4* genomic sequence by PCR. PCR product amplified by **A.** primer pairs AL1F and AL1R; **B.** primer pairs AL2F and AL2R; **C.** primer pairs AL3F and AL3R; **D.** primer pairs AL4F and AL4R; **E.** primer pairs AL5F and AL5R; **F.** primer pairs AL6F and AL6R. The length of marker fragments is 2000, 1000, 750, 500, and 100 bp for A to F, except for C of which marker fragments is 3000, 2000, 1000, 750, 500, 250, and 100 bp.

Expression profile of porcine *ANGPTL4* mRNA

Using SYBR Green real-time PCR, we detected mRNA expression of porcine *ANGPTL4* in the backfat of F1 hybrids and their parents. *GAPDH* was used to normalize the expression level of *ANGPTL4*. The relative quantification showed that *ANGPTL4* mRNA was upregulated in the western commercial breed, Large White pigs, compared with Chinese indigenous pigs, Meishan and their F1 crossbreeds (Figure 4A).

Real-time analysis was also performed to determine the spatial expression of *ANGPTL4* in various tissues (Figure 4B). Porcine *ANGPTL4* was expressed in the ovary, spleen, kidney, backfat, liver, lung, uterus, stomach, and small intestine in high to low abundance, respectively, whereas it was weakly expressed in the heart and longissimus dorsi.

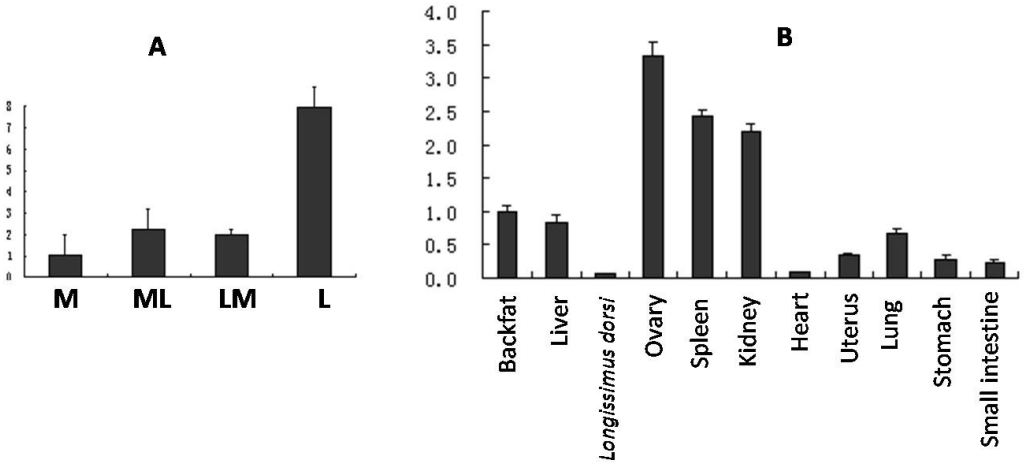

Figure 4. mRNA relative quantitative expression of porcine *ANGPTL4*. Error bars indicate the SD (N = 4) of relative mRNA expression levels of three isoforms to *GAPDH*, determined by real-time quantitative PCR. **A.** mRNA expression of *ANGPTL4* in backfat of Large White and Meishan pigs and their F1 hybrids. The value of *ANGPTL4* in Meishan pigs was arbitrarily set to 1. **B.** Tissue distribution of porcine *ANGPTL4* including backfat, liver, longissimus dorsi, ovary, spleen, kidney, heart, uterus, lung, stomach and small intestine. The value of *ANGPTL4* in backfat was arbitrarily set to 1.

Genotyping and association analysis of *ANGPTL4* PCR-SSCP G2737A

ANGPTL4 is so conserved that only one mutation, G2737A located in the third intron, was found. Association analysis between this polymorphism of porcine *ANGPTL4* and certain traits was performed (Table 2). The *ANGPTL4* genotypes showed a significant effect on water moisture of the longissimus dorsi ($P < 0.05$), meat marbling of the longissimus dorsi ($P < 0.05$), IMF of the longissimus dorsi ($P < 0.05$), and pH of the longissimus dorsi ($P < 0.05$). This site seemed to be significantly ($P < 0.05$) additive in its action on water moisture, IMF, and pH, whereas it had significantly dominant effects on meat marbling ($P < 0.05$). In addition, allele A was associated with increased IMF content of the longissimus dorsi.

Table 2. Association analysis of porcine *ANGPTL4* PCR-SSCP G2737A genotypes with meat quality trait.

Traits	Genotype			Genetic effect	
	AA (58)	AB (92)	BB (30)	Addictive	Dominance
Intramuscular fat at longissimus dorsi	3.841 ± 0.060^{a}	3.738 ± 0.048^{b}	3.627 ± 0.083^{ab}	0.106 ± 0.051*	0.002 ± 0.035
pH at longissimus dorsi	6.322 ± 0.017^{a}	6.366 ± 0.014^{b}	6.328 ± 0.024^{ab}	-0.003 ± 0.015*	0.020 ± 0.010
pH at biceps femoris	6.400 ± 0.012	6.423 ± 0.010	6.403 ± 0.017	-0.001 ± 0.010	0.011 ± 0.007
Water holding Capacity	91.114 ± 0.311	91.007 ± 0.247	90.669 ± 0.430	0.223 ± 0.265	0.058 ± 0.182
Water moisture at longissimus dorsi	73.549 ± 0.098^{a}	73.750 ± 0.07^{b}	73.897 ± 0.135^{ab}	-0.174 ± 0.084*	0.014 ± 0.057
Meat marbling at longissimus dorsi	3.576 ± 0.022^{ab}	3.537 ± 0.017^{a}	3.622 ± 0.031^{b}	-0.022 ± 0.019	-0.031 ± 0.013*
Meat marbling at biceps femoris	4.083 ± 0.022	4.061 ± 0.017	4.034 ± 0.031	0.024 ± 0.019	0.001 ± 0.013
Meat color value at longissimus dorsi	18.863 ± 0.188	18.842 ± 0.149	19.249 ± 0.260	-0.097 ± 0.075	-0.047 ± 0.051
Meat color value at biceps femoris	17.918 ± 0.088	17.922 ± 0.070	18.113 ± 0.122	0.024 ± 0.019	0.001 ± 0.013

Data are reported as means ± SE. Values in each line with different lower case superscripts are significantly different at $P < 0.05$. *$P < 0.01$ for other significant differences.

DISCUSSION

ANGPTL3 and ANGPTL4, two members of the angiopoietin-like protein family, are secreted proteins that regulate triglyceride (TG) metabolism in part through their capacity to inhibit the activity of LPL (Koishi et al., 2002; Shimizugawa et al., 2002; Yoshida et al., 2002) and promote white adipose tissue lipolysis (Kersten, 2005; Hato et al., 2008). LPL is known as an endothelium-associated enzyme that catalyzes the hydrolysis of lipoprotein TG and promotes fatty acid storage in the white adipose tissue (Mead et al., 2002; Merkel et al., 2002). This activity is obviously counterbalanced by that of LPL. Therefore, reducing ANGPTL4 activity should promote TG storage in white adipose tissue, whereas increasing it would activate lipolysis. Overexpression of *Angptl4* in mouse white adipose tissue revealed dramatically reduced TG storage and increased levels of plasma TG and glycerol (Mandard et al., 2006; Koliwad et al., 2009), whereas mice lacking the gene (*Angptl4*$^{-/-}$) showed decreased plasma TG levels (Bäckhed et al., 2004). In addition, a recent large population-based study revealed sequence variations in human *ANGPTL4* that are correlated to the loss of function and reduced plasma TG levels (Romeo et al., 2007). In summary, all of these studies support the suggestion that ANGPTL4 plays an important role in lipid metabolism. In the present study, the mRNA expression of *ANGPTL4* in Large White pigs was found to be higher than that in Meishan pigs and their hybrids, which was consistent with the fact that Large White pigs showed a lower fat percentage compared with Chinese indigenous breeds. In this study, the spatial expression of *ANGPTL4* exhibited a widespread distribution in various pig tissues, which was also observed in mice (Kersten et al., 2000; Kim et al., 2000). In mice, *ANGPTL4* was expressed primarily in the adipose tissue, placenta, and liver (Yoon et al., 2000), as well as in the pituitary gland and intestine (Bäckhed et al., 2004; Wiesner et al., 2004). Conversely, *ANGPTL4* was predominantly expressed in the ovary, spleen, and kidney in pigs in the present study. These results indicate that ANGPTL4 not only regulates lipid metabolism in adipose tissue, but also plays regulatory roles in other tissues with blood vessels.

Due to the significant role of ANGPTL4 in lipid metabolism, we propose *ANGPTL4* as a candidate for marker-assisted selection in pig breeding. The *ANGPTL4* genotypes in this study showed a significant effect on water moisture of the longissimus dorsi, meat marbling of the longissimus dorsi, IMF of the longissimus dorsi, and pH of the longissimus dorsi ($P < 0.05$). The polymorphic site seemed to be significantly ($P < 0.05$) additive in its action on wa-

ter moisture, IMF, and pH, whereas it showed significant dominant effects on meat marbling ($P < 0.05$). In addition, allele A showed an effect of increasing IMF content of the longissimus dorsi. Therefore, this locus can be potentially considered as a marker for IMF traits. Although this locus could likely be used for increasing IMF content, more assays and data are needed to further validate the results of the present study.

In summary, we isolated and characterized the porcine *ANGPTL4*. The data presented here will provide a biochemical and structural basis for future studies on porcine ANGPTL4 function, and will potentially lead to a better understanding of porcine ANGPTL4.

ACKNOWLEDGMENTS

Research supported by the National Natural Science Foundation of China (#31000996), the International Foundation for Science (#B/4534-1), the Specialized Research Fund for the Doctoral Program of Higher Education (#200805041012), and the Fundamental Research Funds for the Central Universities (#2009QC006).

REFERENCES

Bäckhed F, Ding H, Wang T, Hooper LV, et al. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad. Sci. U. S. A.* 101: 15718-15723.

Breathnach R, Benoist C, O'Hare K, Gannon F, et al. (1978). Ovalbumin gene: evidence for a leader sequence in mRNA and DNA sequences at the exon-intron boundaries. *Proc. Natl. Acad. Sci. U. S. A.* 75: 4853-4857.

Caserta F, Tchkonia T, Civelek VN, Prentki M, et al. (2001). Fat depot origin affects fatty acid handling in cultured rat and human preadipocytes. *Am. J. Physiol. Endocrinol. Metab.* 280: E238-E247.

Hato T, Tabata M and Oike Y (2008). The role of angiopoietin-like proteins in angiogenesis and metabolism. *Trends Cardiovasc. Med.* 18: 6-14.

Hocquette JF, Gondret F, Baéza E, Médale F, et al. (2010). Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers. *Animal* 4: 303-319.

Hovenier R, Kanis E and Verhoeven JA (1993). Repeatability of taste panel tenderness scores and their relationships to objective pig meat quality traits. *J. Anim. Sci.* 71: 2018-2025.

Kersten S (2005). Regulation of lipid metabolism via angiopoietin-like proteins. *Biochem. Soc. Trans.* 33: 1059-1062.

Kersten S, Mandard S, Tan NS, Escher P, et al. (2000). Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene. *J. Biol. Chem.* 275: 28488-28493.

Kim I, Kim HG, Kim H, Kim HH, et al. (2000). Hepatic expression, synthesis and secretion of a novel fibrinogen/angiopoietin-related protein that prevents endothelial-cell apoptosis. *Biochem. J.* 346 (Pt 3): 603-610.

Koishi R, Ando Y, Ono M, Shimamura M, et al. (2002). Angptl3 regulates lipid metabolism in mice. *Nat. Genet.* 30: 151-157.

Koliwad SK, Kuo T, Shipp LE, Gray NE, et al. (2009). Angiopoietin-like 4 (ANGPTL4, fasting-induced adipose factor) is a direct glucocorticoid receptor target and participates in glucocorticoid-regulated triglyceride metabolism. *J. Biol. Chem.* 284: 25593-25601.

Livak KJ and Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods* 25: 402-408.

Mandard S, Zandbergen F, van Straten E, Wahli W, et al. (2006). The fasting-induced adipose factor/angiopoietin-like protein 4 is physically associated with lipoproteins and governs plasma lipid levels and adiposity. *J. Biol. Chem.* 281: 934-944.

Mead JR, Irvine SA and Ramji DP (2002). Lipoprotein lipase: structure, function, regulation, and role in disease. *J. Mol. Med.* 80: 753-769.

Merkel M, Eckel RH and Goldberg IJ (2002). Lipoprotein lipase: genetics, lipid uptake, and regulation. *J. Lipid Res.* 43: 1997-2006.

Oike Y, Akao M, Kubota Y and Suda T (2005). Angiopoietin-like proteins: potential new targets for metabolic syndrome therapy. *Trends Mol. Med.* 11: 473-479.

Pethick DW, Harper GS and Oddy VH (2004). Growth, development and nutritional manipulation of marbling in cattle: A review. *Aust. J. Exp. Agric.* 44: 705-715.

Ren ZQ, Xiong YZ, Deng CY, Zuo B, et al. (2005). Age-dependent changes of differential gene expression profile in backfat tissue between hybrids and parents in pigs. *Asian-Aust. J. Anim. Sci.* 18: 682-685.

Romeo S, Pennacchio LA, Fu Y, Boerwinkle E, et al. (2007). Population-based resequencing of ANGPTL4 uncovers variations that reduce triglycerides and increase HDL. *Nat. Genet.* 39: 513-516.

Shimizugawa T, Ono M, Shimamura M, Yoshida K, et al. (2002). ANGPTL3 decreases very low density lipoprotein triglyceride clearance by inhibition of lipoprotein lipase. *J. Biol. Chem.* 277: 33742-33748.

Sukonina V, Lookene A, Olivecrona T and Olivecrona G (2006). Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue. *Proc. Natl. Acad. Sci. U. S. A.* 103: 17450-17455.

Wiesner G, Morash BA, Ur E and Wilkinson M (2004). Food restriction regulates adipose-specific cytokines in pituitary gland but not in hypothalamus. *J. Endocrinol.* 180: R1-R6.

Wood JD, Enser M, Fisher AV, Nute GR, et al. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Sci.* 78: 343-358.

Yoon JC, Chickering TW, Rosen ED, Dussault B, et al. (2000). Peroxisome proliferator-activated receptor gamma target gene encoding a novel angiopoietin-related protein associated with adipose differentiation. *Mol. Cell. Biol.* 20: 5343-5349.

Yoshida K, Shimizugawa T, Ono M and Furukawa H (2002). Angiopoietin-like protein 4 is a potent hyperlipidemia-inducing factor in mice and inhibitor of lipoprotein lipase. *J. Lipid Res.* 43: 1770-1772.