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

Molecular characterization and tissue expression profile analysis of the porcine *JAZF1* gene

H. Yang[1,2], J. He[1], X.L. Xu[2], J. Jiang[1], C.Q. He[1] and H.M. Ma[1]

[1]College of Animal Science and Technology of Hunan Agricultural University, Changsha, China
[2]College of Life Sciences and Resource Environment of Yichun University, Yichun, China

Corresponding author: H.M. Ma
E-mail: mahaiming2000@163.com

Genet. Mol. Res. 14 (1): 542-551 (2015)
Received January 15, 2014
Accepted August 6, 2014
Published January 26, 2015
DOI http://dx.doi.org/10.4238/2015.January.26.9

ABSTRACT. A recent study indicated that the *JAZF1* gene was related to lipid metabolism by regulating the level of gene expression in humans and mice. In order to investigate whether *JAZF1* gene expression was associated with fat deposition in pig, we cloned the full-coding region of the *JAZF1* gene (GenBank accession No. KF307636) from porcine longissimus dorsi. Results showed that the open reading frame of *JAZF1* covered 732 bp and encoded 243 amino acids. Multiple alignment of isoform sequences revealed that the deduced amino acid sequence of JAZF1 had a high degree of sequence similarity to other vertebrates, indicating that it was highly conserved during evolution. Bioinformatic analysis indicated that pig JAZF1 contained 23 phosphorylation sites and 19 glycosyl sites. JAZF1 was predicted to have 3 ZnF-C_2H_2 and 2 low-complexity domains. The *JAZF1* mRNA expression pattern indicated that *JAZF1* mRNA expression level in the liver was significantly different in 2 divergent breeds ($P < 0.05$). This article perhaps provided an

important experimental basis for further research on the mechanisms of lipid metabolism and fat deposition in pigs.

Key words: Shaziling pig; *JAZF1* gene; Tissue expression

INTRODUCTION

Juxtaposed with another zinc finger gene 1 (*JAZF1*) is a newly identified gene that is associated with prostate cancer, diabetes mellitus, and endometrial cancer (Koontz et al., 2001; Thomas et al., 2008; Zeggini et al., 2008). The *JAZF1* gene encodes a basic protein with a molecular mass of 27.1 kDa that contains 2 zinc finger motifs and acts as a repressor of DR1-dependent transcriptional activation by the testicular orphan nuclear receptor 4 (TR4) (Nakajima et al., 2004). The TR4 receptor is a regulator of transcription and controls many important physiological functions (Hirose et al., 1994; Lee and Chang, 1995). A study revealed that *JAZF1* overexpression resulted in high blood pressure, electrocardiogram abnormalities, and mitochondrial defects (Bae et al., 2011). Recently, studies also showed that the *JAZF1* gene reduced lipid synthesis and increased lipolysis by regulating the level of expression of genes that were correlated with fat metabolism in mice (Li et al., 2011). Like the *JAZF1* gene, many genes associated with fat metabolism were applied to pig breeding as candidate genes to improve meat quality. Pork meat quality is an important trait for breeding in the porcine industry (Fiedler et al., 2003). One of the most important parameters affecting meat quality is intramuscular fat (IMF) content, which is a key determinant of organoleptic evaluation and the nutritional value of pork (Calkins and Hodgen, 2007; Oury et al., 2009). An increased IMF content was associated with higher juiciness and flavor scores (Fernandez et al., 1999a). Because of the positive relationship between IMF content and meat tenderness (Wood et al., 2004; Fortin et al., 2005; Chmurzyńska, 2006), numerous genes associated with fat metabolism in humans and mice have been applied to pig breeding to improve IMF content; these genes include *NUDT6*, *FABP3*, *H-FABP*, and *FTO* (Fontanesi et al., 2009; Li et al., 2010; Ding et al., 2011; Sun et al., 2012).

Studies in humans and mice indicate that *JAZF1* could be considered as a candidate gene for improving the IMF content of pork because of its role in regulating the gene expression. However, the association between the *JAZF1* gene and IMF content in pigs has not been reported. This study mainly aimed to determine the open reading frame (ORF) of cDNAs of the *JAZF1* gene and investigate its expression levels in 10 tissues of 2 pig breeds. We expect to provide a further data to reveal the function of the porcine *JAZF1* gene.

MATERIAL AND METHODS

Sample collection

The longissimus dorsi muscle samples were collected from 3 castrated male Shaziling pigs that were 25 days old. Total RNA was extracted from these longissimus dorsi muscle samples using the total RNA extraction kit (Column Animal RNAout, China) and was used in polymerase chain reaction (PCR) and quantitative real-time PCR.

The longissimus dorsi, crureus, lung, liver, pancreas, intestine, cecum, heart, spleen, and kidney samples were collected from three 25-day-old Shaziling pigs and three 25-day-old Yorkshire pigs, immediately snap frozen by liquid nitrogen, and stored at -80°C until used. Total RNA was extracted from these tissues using the total RNA extraction kit, and these RNA samples were used in real-time PCR for tissue expression analysis. DNase I treatment of total RNA was performed before the first-strand cDNA synthesis.

RNA extraction, reverse transcription, cloning, and sequencing

The total RNA was extracted from the longissimus dorsi of a Shaziling pig using the total RNA extraction kit (Column Animal RNAout) in accordance with manufacturer instructions. RNA quality and concentrations were evaluated. The first-strand cDNA was produced using the SUPERSCRIPT II RT Reverse Transcription System kit (Invitrogen, USA) in accordance with the manufacturer protocol. According to the human *JAZF1* mRNA sequence (GenBank accession: NM_175061), a pair of JAZF1 primers (Table 1) was designed to obtain a fragment of the coding sequence (CDS) of the pig *JAZF1* gene. PCR was conducted under the following conditions: 94°C for 5 min; 32 cycles of 94°C for 20 s, 54°C for 25 s, and 72°C for 30 s; and 72°C for 5 min. After gel purification, the reverse transcription PCR products were cloned into the pMD19-T vector (Takara, Japan) and sequenced.

Table 1. Primer sequences used in this study.

Primer	Nucleotide sequence (5'-3')	Size (bp)	Application
JAZF1-CDS	F: GCTAAGAATGGTCACAGAAC R: TTATGTAAGGCATGGTAACC	620	Coding sequence
JAZF1-qPCR	F: ACCACTGCTTGAGGTGCTACA R: AATGTCCGCAACTCAGTCAGG	102	Coding sequence
GAPDH	F: ATTTGGCTACAGCAACAGGGT R: AAGTCAGGAGATGCTCGGTGT	172	Coding sequence
GSP3 UPM	F: TCAATTTCCATCCCCCAGTGTCGGCT R: CTAATACGACTCACTATAGGGC	386	3'-RACE
GSP5 AUAP	R: CGAATCTGTGTTCTGTGACCA F: GGCCACGCGTCGACTAGTAC	684	5'-RACE

Rapid amplification of cDNA ends (RACE)

To obtain the full-length porcine *JAZF1* cDNA, 2 pairs of primers were designed to amplify the 5' and 3' ends of the *JAZF1* gene and obtain the CDS of the pig *JAZF1* gene. 5'-RACE and 3'-RACE PCR were performed according to the instruction of the SMART™ RACE cDNA Amplification Kit (BD Science, USA). Gene-specific primers, universal primer, and abridged universal amplification primer sequences are listed in Table 1. The PCR products were separated on a 1.5% agarose gel. After gel purification, the reverse transcription PCR products were cloned into the pMD19-T vector (Takara) and sequenced. Through the DNAStar Lasergene 7.1 software, the full-length cDNA sequence was achieved. The sequence homology was evaluated with the National Center for Biotechnology Information Basic Local Alignment Search Tool database.

Bioinformatic analyses

The cDNA sequence analysis and the amino acid sequence deduction were performed with the Expert Protein Analysis System (http://www.expasy.org/). The amino acid sequences of JAZF1 were aligned using CLUSTALW version 2.0, and the protein domain features of JAZF1 were predicted using the simple modular architecture tool (http://smart.embl-heidelberg.de/). A phylogenetic chart was constructed with the full-length cDNA sequences of previously published *JAZF1* genes using the neighbor-joining method with MEGA version 5.0.

Real-time quantitative PCR (qPCR) analysis

To evaluate the expression of porcine *JAZF1* in different tissues, RNA was isolated from 10 mg tissue using the RNA extraction kit (Qiagen, China) following manufacturer instructions. Total cDNA was generated with QuantiTect Transcription Kit (Qiagen). SYBR Green primers were designed using the Premier 5.0 software (Table 1). qPCR was carried out in a real-time PCR System (ABI, USA). The results were calculated with the relative quantification method with glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as the reference gene. *JAZF1* expression levels were calculated by the $2^{-\Delta\Delta Ct}$ comparative method. qPCR amplifications were carried out in a final volume of 20 µL comprising 10 µL 2X qPCR Mix (ABI), 1 µL of each primer (10 µM), 1 µL template cDNA, and 8 µL ddH_2O with the following program: 95°C for 2 min and 40 cycles of 95°C for 10 s, 60°C for 40 s, and 72°C for 30 s.

RESULTS

Sequence analysis and comparison of amino acid sequence homology among animal JAZF1

The cDNA of the *JAZF1* gene from a Shaziling pig was amplified by reverse transcription PCR. The full-length cDNA of *JAZF1* with 1098 bp was obtained successfully by the RACE method. The cDNA sequences and the deduced amino acid sequence are shown in Figure 1. The nucleotide sequence of *JAZF1* possessed an ORF of 732 bp, which encoded a protein of 243 amino acids. The molecular weight of JAZF1 was about 27.0 kDa, with a calculated isoelectric point of 8.63.

By analyzing the primary structure, JAZF1 comprised 243 amino acids and contained a predicted transmembrane domain, 23 phosphorylation sites (Ser18, Thr2, and Tyr3), and 19 glycosyl sites. The domain structure of JAZF1 included 3 ZnF-C_2H_2 and 2 low-complexity domains (Figure 2). The ZnF-C_2H_2 domain was made up of 72 amino acids, and the low-complexity region was made up of 49 amino acids (Table 2).

We aligned the pig JAZF1 amino acid sequence with that of other mammals. As shown in Figure 3, the 100% similarity between the porcine JAZF1 and other animals except *Mus musculus* at the amino acid level indicated that the gene could be defined as the pig *JAZF1* gene. These results identified the high conservation of *JAZF1* and the close relationship of the animals. These results also indicated that the *JAZF1* gene might have the same biological function in different animals.

```
agccgacaccaggggggctctcgatgtagcaccatgacaggcatcgccgccgcctccttc
                                    M  T  G  I  A  A  A  S  F
ttctccaatacctgccgattcgggggctgcggactccacttccccaccctggccgacctc
 F  S  N  T  C  R  F  G  G  C  G  L  H  F  P  T  L  A  D  L
atcgagcacatcgaggacaaccacatcgatacagatccacgtgttttagaaaaacaagaa
 I  E  H  I  E  D  N  H  I  D  T  D  P  R  V  L  E  K  Q  E
ttacagcagccaacctatgttgccctcagttacattaatagattcatgacagacgctgcc
 L  Q  Q  P  T  Y  V  A  L  S  Y  I  N  R  F  M  T  D  A  A
cgccgggagcaggagtccctgaagaagaagattcagccgaagctgtcgctgaccttgtcc
 R  R  E  Q  E  S  L  K  K  K  I  Q  P  K  L  S  L  T  L  S
agctctgtgtctcgcggaaatgtgtccactccgccacgtcacagcagcggaagccttact
 S  S  V  S  R  G  N  V  S  T  P  P  R  H  S  S  G  S  L  T
ccccccgtgaccccgcccatcaccccctcctcttcattccgcagcagcaccccaacaggc
 P  P  V  T  P  P  I  T  P  S  S  S  F  R  S  S  T  P  T  G
agtgagtacgacgaggaggaggtggattacgaggagtcggacagcgatgagtcctggacc
 S  E  Y  D  E  E  E  V  D  Y  E  E  S  D  S  D  E  S  W  T
acagagagcgccatcagctccgaagccatcctcagctccatgtgcatgaacggaggggag
 T  E  S  A  I  S  S  E  A  I  L  S  S  M  C  M  N  G  G  E
gagaagcctttcgcctgcccggttcctggatgcaaaaagagatacaagaatgtgaatggc
 E  K  P  F  A  C  P  V  P  G  C  K  K  R  Y  K  N  V  N  G
ataaagtaccatgctaagaatggtcacagaacacagattcgtgtccgcaaaccattcaaa
 I  K  Y  H  A  K  N  G  H  R  T  Q  I  R  V  R  K  P  F  K
tgccgctgtggtaagagttacaagacagctcagggcctgaggcaccacacaatcaatttc
 C  R  C  G  K  S  Y  K  T  A  Q  G  L  R  H  H  T  I  N  F
catcccccagtgtcggctgagattatcaggaagatgcagcaataacatgctggtcataac
 H  P  P  V  S  A  E  I  I  R  K  M  Q  Q  *
tgtgccaagaaatcctcaccggcagttgctgattttgaaaacagccacctttttcagggg
aagcattcagcaaccctttaaagaattaaatgcatgctttaaatttttttctgtaattttg
gaatgatgtatctttgtagagttaatgattttgtacatttgcacatgtaatcatcatacc
cattttcattacttttgatataaggtgctaaacaaacaaacaaacaaaaaagctctaggtt
cttcagcacatttcccccaaaacaaaataaaaattgagggcatgttgcaaaaaaaaaaaa
aaaaaaaaaaaaaaaaa
```

Figure 1. Complete cDNA sequence and predicted amino acid sequence of the *JAZF1* gene. ATG, start codon; TAA, stop codon; asterisk indicates the stop codon.

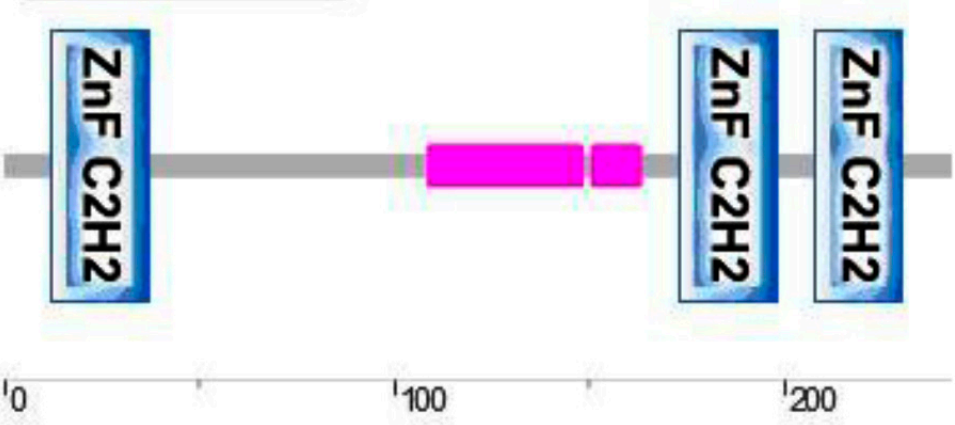

Figure 2. JAZF1 conservative structure domain analysis.

Table 2. Predicted domains, repeats, motifs, and features.

Name	Start	End	Length (amino acids)
Zn-C_2H_2	12	37	25
Low complexity	109	147	38
Low complexity	151	162	11
Zn-C_2H_2	173	198	25
Zn-C_2H_2	208	230	22

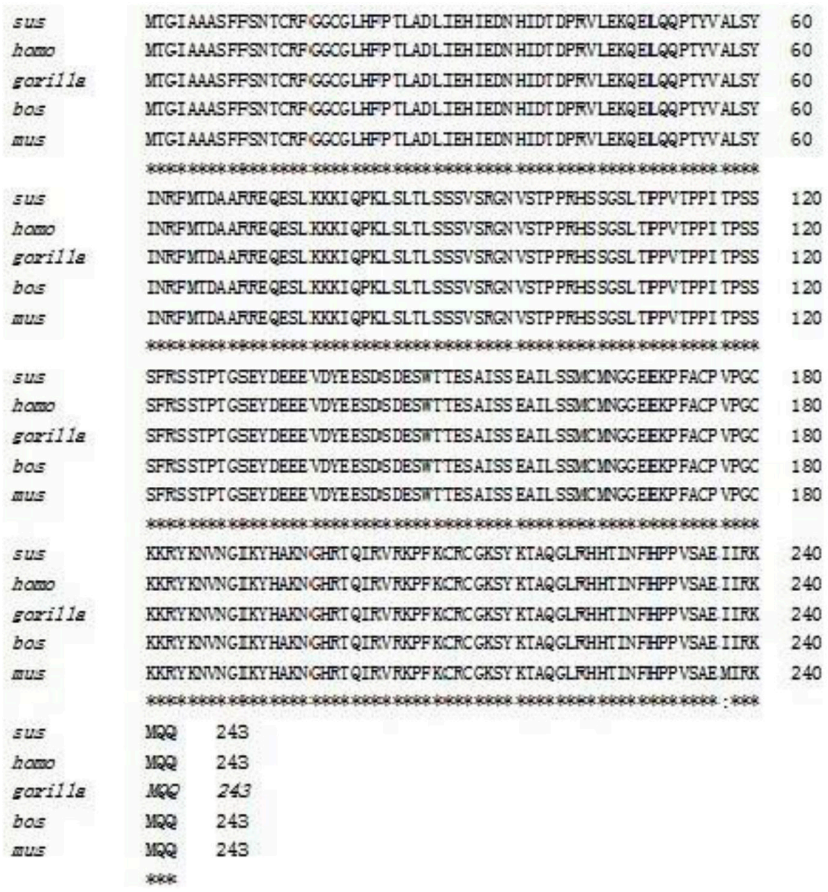

Figure 3. Alignment of the protein encoded by the porcine *JAZF1* gene and the JAZF1 amino acid sequence from *Homo sapiens*, *Gorilla gorilla*, *Bos taurus*, and *Mus musculus*.

Phylogenetic analysis

A phylogenetic tree (Figure 4) was constructed from the multiple alignments of the deduced amino acid sequences of porcine JAZF1 with the corresponding isoform sequences of other animals. The *Homo sapiens* JAZF1 formed a cluster with the *Pan troglodytes*, *Macaca mulatta*, pig, and sheep; the *Bos taurus* JAZF1 formed another cluster; and *Mus musculus* and silurana formed a third cluster.

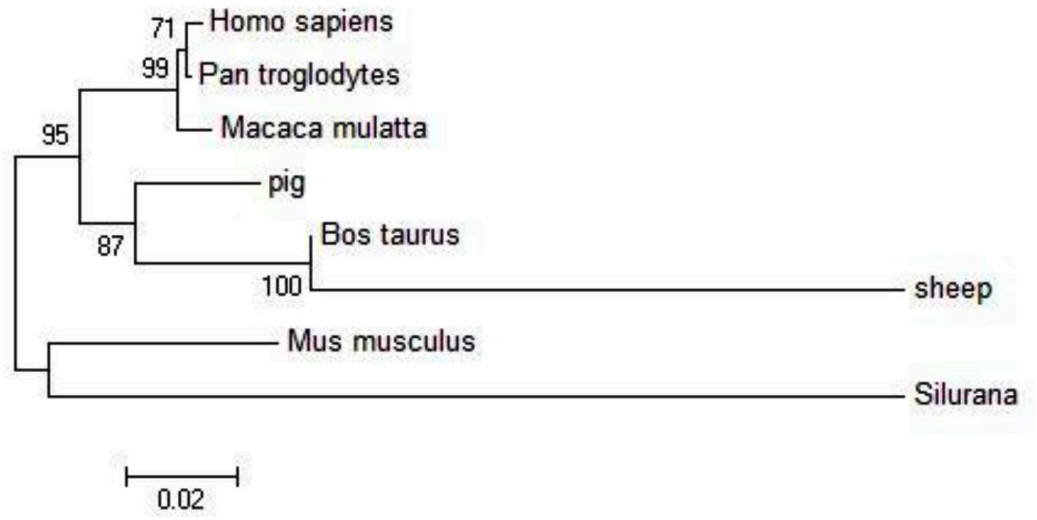

Figure 4. Phylogenetic relationship among the *JAZF1* gene of pig and other species.

Tissue expression pattern of the porcine *JAZF1* gene

The expression pattern of the *JAZF1* gene was analyzed in 10 different tissues of Shaziling and Yorkshire pigs by qPCR (Figure 5). The results indicated that the mRNA of the *JAZF1* gene was expressed at a low level in lungs, kidney, cecum, intestine, and crureus; at a moderate level in heart, liver, and longissimus dorsi tissues; and at the highest level in spleen and pancreas.

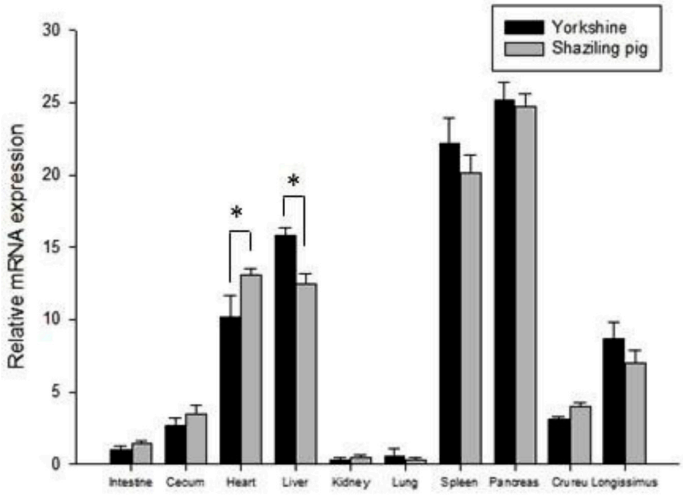

Figure 5. Expression profile of the *JAZF1* gene in 10 different tissues of the Shaziling and Yorkshire breeds. RNA samples were normalized using the *GAPDH* gene. Bars indicate the standard deviation of the mean. $P < 0.05$ is marked with an asterisk.

DISCUSSION

The pig is an important domestic animal. Pork production and consumption play important roles in the development of the economy (de Almeida and Bendixen, 2012). Recently, improvement in meat quality has become one of the most important porcine breeding goals (Lee et al., 2012). *JAZF1*, also known as *TIP27*, which encodes a transcriptional repressor of NR2C2 (Nakajima et al., 2004), is involved in regulating the metabolism of growth and affects variation in human height (Johansson et al., 2009). To investigate the function of *JAZF1* in pigs, we obtained the full-cDNA sequence of *JAZF1* by RACE. The full-cDNA sequence of the *JAZF1* gene was 1098 bp, and the ORF was 732 bp, encoding 243 amino acids. The molecular weight of JAZF1 was about 27.0 kDa, and it contained 3 putative zinc finger motifs and 2 low-complexity regions. Multiple alignment of JAZF1 with the corresponding isoform sequences of other vertebrates showed strong amino acid conservation (Figure 3), which indicated that JAZF1 might have parallel biological functions. Based on the deduced amino acid sequence of porcine JAZF1 with the corresponding isoform sequences of other vertebrates, a phylogenetic tree (Figure 4) was constructed. The phylogenetic analysis provided evidence that the closest isoforms to porcine JAZF1 were those of sheep and cattle.

The *JAZF1* mRNA expression distribution in 10 tissues showed that expression in spleen, pancreas, liver, and longissimus dorsi muscle was higher than other tissues; this result was consistent with those of previous studies. We also found that *JAZF1* mRNA expression in liver and heart was significantly different in 2 divergent breeds ($P < 0.05$). The transcript abundance in liver and longissimus dorsi was higher in the Yorkshire breed than in the Shaziling breed, which may be related to reduced lipid synthesis and increased lipolysis by downregulating the levels of fatty acid synthetase mRNA expression (Li et al., 2011).

A study in humans and mice revealed that *JAZF1* was associated with diabetes mellitus (Grarup et al., 2008) and had an effect on the decrease of fatty acid synthesis by regulating the transcription factor SREBP1, which plays important roles in the control of fatty acid metabolism and adipogenesis (Stoeckman and Towle, 2002). In order to investigate the molecular characterization and expression level of the *JAZF1* gene in different tissues in pig, we cloned the full-length cDNA of *JAZF1* and performed qPCR, which could provide important contributions to future studies of the molecular mechanisms that lead to reduced fatty acid synthesis and increased lipolysis in pig.

Improving meat quality, especially the IMF content of pork, is of major importance in the meat industry. The IMF content has a significant effect on meat palatability and tenderness (Fernandez et al., 1999b; Tong et al., 2008; Hausman et al., 2009). One possible cause for the influence could be that the infiltration of the IMF within the perimysium connective tissue weakened the cross-linkage between collagen fibers, reducing the force required to breakdown the connective tissue (Essén-Gustavsson et al., 1994). Few studies have suggested that the association between the IMF content and tenderness might be deciphered by the fat cell expansion in the perimysium, which might open the muscle structure by forcing the muscle bundles apart (Wood, 1990). Recently, most of studies on IMF have focused on the mechanisms of lipogenesis (Morcuende et al., 2007; Zhao et al., 2009), and numerous candidate genes associated with IMF deposition had been identified (Cánovas et al., 2010; Serão et al., 2011; Tyra and Ropka-Molik, 2011), which may improve our ability to effectively enhance the IMF content in pork and other livestock.

ACKNOWLEDGMENTS

Research supported by the National High Technology Research and Development Program of China (Grant #2011AA 1003 04), the Provincial Natural Science Foundation of Hunan (Grant #13JJ1021), and the Key Foundation of Education Department in Hunan (Grant #12A060).

REFERENCES

Bae KB, Kim MO, Yu DH, Shin MJ, et al. (2011). Overexpression of Jazf1 induces cardiac malformation through the upregulation of pro-apoptotic genes in mice. *Transgenic Res*. 20: 1019-1031.

Calkins CR and Hodgen JM (2007). A fresh look at meat flavor. *Meat Sci*. 77: 63-80.

Cánovas A, Quintanilla R, Amills M and Pena R (2010). Muscle transcriptomic profiles in pigs with divergent phenotypes for fatness traits. *BMC Genomics* 11: 372.

Chmurzyńska A (2006). The multigene family of fatty acid-binding proteins (FABPs): function, structure and polymorphism. *J. Appl. Genet*. 47: 39-48.

de Almeida AM and Bendixen E (2012). Pig proteomics: a review of a species in the crossroad between biomedical and food sciences. *J. Proteomics* 75: 4296-4314.

Ding X, Ding Y, Zhang C, Huang L, et al. (2011). Polymorphisms and sequencing analysis in 5'upstream and intron 2 region of *H-FABP* gene in Anhui Wannan black pig population. *J. Anim. Vet. Adv*. 10: 1568-1572.

Essén-Gustavsson B, Karlsson A, Lundström K and Enfält AC (1994). Intramuscular fat and muscle fibre lipid contents in halothane-gene-free pigs fed high or low protein diets and its relation to meat quality. *Meat Sci*. 38: 269-277.

Fernandez X, Monin G, Talmant A, Mourot J, et al. (1999a). Influence of intramuscular fat content on the quality of pig meat - 1. Composition of the lipid fraction and sensory characteristics of m. longissimus lumborum. *Meat Sci*. 53: 59-65.

Fernandez X, Monin G, Talmant A, Mourot J, et al. (1999b). Influence of intramuscular fat content on the quality of pig meat - 2. Consumer acceptability of m. longissimus lumborum. *Meat Sci*. 53: 67-72.

Fiedler I, Nürnberg K, Hardge T, Nürnberg G, et al. (2003). Phenotypic variations of muscle fibre and intramuscular fat traits in longissimus muscle of F_2 population Duroc×Berlin Miniature Pig and relationships to meat quality. *Meat Sci*. 63: 131-139.

Fontanesi L, Scotti E, Buttazzoni L, Davoli R, et al. (2009). The porcine fat mass and obesity associated (FTO) gene is associated with fat deposition in Italian Duroc pigs. *Anim. Genet*. 40: 90-93.

Fortin A, Robertson WM and Tong AKW (2005). The eating quality of Canadian pork and its relationship with intramuscular fat. *Meat Sci*. 69: 297-305.

Grarup N, Andersen G, Krarup NT, Albrechtsen A, et al. (2008). Association testing of novel type 2 diabetes risk alleles in the *JAZF1*, *CDC123*/*CAMK1D*, *TSPAN8*, *THADA*, *ADAMTS9*, and *NOTCH2* loci with insulin release, insulin sensitivity, and obesity in a population-based sample of 4,516 glucose-tolerant middle-aged Danes. *Diabetes* 57: 2534-2540.

Hausman GJ, Dodson MV, Ajuwon K, Azain M, et al. (2009). Board-invited review: the biology and regulation of preadipocytes and adipocytes in meat animals. *J. Anim. Sci*. 87: 1218-1246.

Hirose T, Fujimoto W, Tamaai T, Kim KH, et al. (1994). TAK1: molecular cloning and characterization of a new member of the nuclear receptor superfamily. *Mol. Endocrinol*. 8: 1667-1680.

Johansson Å, Marroni F, Hayward C, Franklin CS, et al. (2009). Common variants in the *JAZF1* gene associated with height identified by linkage and genome-wide association analysis. *Hum. Mol. Genet*. 18: 373-380.

Koontz JI, Soreng AL, Nucci M, Kuo FC, et al. (2001). Frequent fusion of the *JAZF1* and *JJAZ1* genes in endometrial stromal tumors. *Proc. Natl. Acad. Sci. U. S. A*. 98: 6348-6353.

Lee EA, Kim JM, Lim KS, Ryu YC, et al. (2012). Effects of variation in porcine *MYOD1* gene on muscle fiber characteristics, lean meat production, and meat quality traits. *Meat Sci*. 92: 36-43.

Lee HJ and Chang C (1995). Identification of human TR2 orphan receptor response element in the transcriptional initiation site of the simian virus 40 major late promoter. *J. Biol. Chem*. 270: 5434-5440.

Li L, Yang Y, Yang G, Lu C, et al. (2011). The role of JAZF1 on lipid metabolism and related genes *in vitro*. *Metabolism* 60: 523-530.

Li X, Kim SW, Choi JS, Lee YM, et al. (2010). Investigation of porcine *FABP3* and *LEPR* gene polymorphisms and

mRNA expression for variation in intramuscular fat content. *Mol. Biol. Rep*. 37: 3931-3939.
Morcuende D, Estévez M, Ramírez R and Cava R (2007). Effect of the IberianxDuroc reciprocal cross on productive parameters, meat quality and lipogenic enzyme activities. *Meat Sci*. 76: 86-94.
Nakajima T, Fujino S, Nakanishi G, Kim YS, et al. (2004). TIP27: a novel repressor of the nuclear orphan receptor TAK1/TR4. *Nucleic Acids Res*. 32: 4194-4204.
Oury MP, Picard B, Briand M, Blanquet JP, et al. (2009). Interrelationships between meat quality traits, texture measurements and physicochemical characteristics of M. rectus abdominis from Charolais heifers. *Meat Sci*. 83: 293-301.
Serão NVL, Veroneze R, Ribeiro AMF, Verardo LL, et al. (2011). Candidate gene expression and intramuscular fat content in pigs. *J. Anim. Breed. Genet*. 128: 28-34.
Stoeckman AK and Towle HC (2002). The role of SREBP-1c in nutritional regulation of lipogenic enzyme gene expression. *J. Biol. Chem*. 277: 27029-27035.
Sun L, Yu S, Wang H, Fan B, et al. (2012). *NUDT6*, the *FGF-2*'s antisense gene, showed associations with fat deposition related traits in pigs. *Mol. Biol. Rep*. 39: 4119-4126.
Thomas G, Jacobs KB, Yeager M, Kraft P, et al. (2008). Multiple loci identified in a genome-wide association study of prostate cancer. *Nat. Genet*. 40: 310-315.
Tong J, Zhu MJ, Underwood KR, Hess BW, et al. (2008). AMP-activated protein kinase and adipogenesis in sheep fetal skeletal muscle and 3T3-L1 cells. *J. Anim. Sci*. 86: 1296-1305.
Tyra M and Ropka-Molik K (2011). Effect of the *FABP3* and *LEPR* gene polymorphisms and expression levels on intramuscular fat (IMF) content and fat cover degree in pigs. *Livest. Sci*. 142: 114-120.
Wood JD (1990). Consequences for meat quality of reducing carcass fatness. In: Reducing fat in meat animals (Wood JD and Fisher AV, eds.). Elsevier, London, 344-397.
Wood JD, Richardson RI, Nute GR, Fisher AV, et al. (2004). Effects of fatty acids on meat quality: a review. *Meat Sci*. 66: 21-32.
Zeggini E, Scott LJ, Saxena R, Voight BF, et al. (2008). Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nat. Genet*. 40: 638-645.
Zhao SM, Ren LJ, Chen L, Zhang X, et al. (2009). Differential expression of lipid metabolism related genes in porcine muscle tissue leading to different intramuscular fat deposition. *Lipids* 44: 1029-1037.