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Molecular Characterization and Tissue Expression of the *FSP27* Gene in Wujin Pigs

[1,2]Hongbin Pan, [2]Sumei Zhao, [2]Jiawei Jiang, [2]Ying Huang, [2]Minghua Yang,
[2]Yongke Sun, [2]Shizheng Gao and [1]Guixin Qin
[1]Institute of Animal Science and Technology,
Jilin Agricultural University, 130118 Jilin, Changchun, China
[2]Yunnan Key Laboratory of Animal Nutrition and Feed Science,
College of Animal Science and Technology, Yunnan Agricultural University,
650201 Kunming, China

Abstract: Fat-Specific Protein 27 (FSP27) could be a potential biomarker promoting neutral lipid storage and thereby a candidate gene for the regulation of the Intramuscular Fat (IMF) deposition in pigs. In this study, the cloning and comparison of the Coding Domain Sequence (CDS) and the deduction of amino acids sequence of *FSP27* gene in longissimus dorsi muscle between Wujin and Landrace pigs suggest that the CDS of *FSP27* gene is 747 bp encoding for 248 amino acids. One silent polymorphism (C→T) occurred in 549th nucleotide in the CDS of the *FSP27* gene in Wujin pigs. Secondary structure analysis of FSP27 deduced amino acids found 67 helixes, 49 strands, 132 coils and 8 transmembrane helices. The FSP27 was localized in the mitochondria. Moreover, The FSP27 contained 6 exons which the sequence lengths was 33, 78, 154, 159, 188 and 682 bp, respectively. The 3'-UTR region of *FSP27* gene could to contain potential regulatory sequences for some miRNA for example hsa-miR-491-5p and hsa-miR-4510. The promoter region of *FSP27* gene exhibited binding sites of AML-1a, NF-κ-β and SRY transcription factors. The expression level of the FSP27 mRNA was significantly higher in longissimus dorsi muscle of Wujin pigs than Landrace pigs ($p<0.05$). The different expression levels of the FSP27 mRNA in longissimus dorsi muscle of pigs may relate to the variation of the IMF eposition.

Key words: *FSP27* gene, Wujin pigs, tissue expression, structure predication, moleculer characterization

INTRODUCTION

In pig production, Intramuscular Fat (IMF) content is one of the determinant factors of meat quality characteristics such as tenderness, juiciness and flavor level (Fernandez *et al*., 1999). IMF content varies in different pig breeds (Sellier, 1998). Particularly, IMF content is higher in the Chinese local pigs than in other commercial pigs (Kinyamu and Ewan, 1994; Yen *et al*., 1991; Young, 1992). The Wujin pigs is one of the Chinese local pig breeds which IMF content was significantly greater than Landrace pig (Ge *et al*., 2008; Zhang *et al*., 2008; Zhao *et al*., 2009).

Porcine IMF deposition is the netto result of the Triglycerid (TG) accumulation in intramuscular adipocytes. Cellular Lipid Droplets (LDs) are dynamic organelles that regulate triglyceride stores in cells (Jambunathan *et al*., 2011). Recent gene targeting studies have revealed that CIDE proteins, especially FSP27 are also important modulators in diverse lipid metabolic pathways such as lipolysis, fatty acid oxidation, VLDL lipidation and lipid droplet growth in adipocytes (Yonezawa *et al*., 2011; Gong *et al*., 2009). FSP27 is a LD-associated protein and plays a unique role in LD dynamics, controlling LD size and lipid storage (Li *et al*., 2009; Matsusue *et al*., 2008; Nian *et al*., 2010). Moreover, research found that Ad-36 could induce lipid droplets in the cultured skeletal muscle cells and this process may be mediated by cell death-inducing DFF45-like effector-C (CIDEC)/FSP27 expression (Wang *et al*., 2010). These biological functions of *FSP27* gene suggest that it may be regarded as a candidate gene for IMF deposition in pigs. However, little information is available about the nucleotide and amino acids sequence and the expression level of the *FSP27* gene in Chinese local pig breeds.

The objective of this study was to clone the Code Domain Sequence (CDS) of *FSP27* gene and compare the nucleotide acids and deduced amino acids sequence between Wujin and Landrace pigs. Furthermore, physiological characteristics and molecular structure were analyzed and tissue expression profi les of FSP27 in Wujin pigs had been performed.

Corresponding Author: Guixin Qin, Institute of Animal Science and Technology, Jilin Agricultural University, Av. Xincheng, 2888 Changchun, 130118 Jilin, China

MATERIALS AND METHODS

All experiment procedures were performed according to the Guide for Animal Care and Use of Laboratory Animals in the Institutional Animal Care and Use Committee of Yunnan Agricultural University. The experimental protocol was approved by the Department Animal Ethics Committee of Yunnan Agricultural University.

Animal and samples: The commercial Wujin and Landrace pigs were supplied by a pig farm of Yunnan Province. The 12 Wujin and 12 Landrace pigs were used. They were supplied with compound feed with clear water available *ad libitum*. The animals were slaughtered in 100 kg weight. The longissimus dorsi muscle was sampled at the last rib which were collected for sequence isolation. Tissue samples of Wujin pigs including longissimus dorsi muscle, heart, liver, spleen, lung, kidney and adipose tissue were collected for expression analysis. Parts of the removed tissue samples were snap frozen in liquid nitrogen and stored at -80°C to be used for RNA extraction.

Measurement of IMF content: The longissimus dorsi muscles were sampled for IMF content evaluation 24 h after slaughtering by following the Soxhlet Petroleum-Ether Extraction Method.

Total RNA extraction and reverse transcript: Total RNA was extracted using the Total RNA Extraction kit (Invitrogen, America). Total RNA concentration was quantified by measuring the absorbance at 260 nm in a photometer (Eppendorf Biophotometer). Ratios of absorption (260/280 nm) of all preparations were between 1.8 and 2.0. Aliquots of RNA samples were subjected to electrophoresis through a 1.4% agarose formaldehyde gel to verify their integrity.

Reverse transcription was performed using 2 μg RNA in a final volume of 25 μL containing 10 units of MMLV reverse transcriptase (Promega, Belgium), 1 mM dNTP mixture (Promega), 40 units of recombinant RNasin ribonuclease inhibitor (Promega) and 0.5 μL of oligo (dT) 18 (Promega) in sterilized water and buffer supplied by the manufacturer. After incubation at 42°C for 60 min, the mixture was heat treated at 95°C for 5 min. cDNA samples were kept in -20°C for detection.

cDNA clone: The Reverse Transcription (RT) reaction mix (2 μL) of longissimus dorsi muscles was used for PCR in a final volume of 25 μL containing 1.5 mM $MgCl_2$, 200 μM dNTP, 1.5 IU Taq polymerase and 50 pmol of the forward and reverse primers. The FSP27 primers were F: 5'GAAACATGGAGCCCAACGC3', R: 5' TCACTGC AGTATCT TTAGACAGGT3' designed on the FSP27 sequence of pig (Accession No. NM_001112689.1). During PCR, samples were heated to 94°C for 3 min followed by 35 cycles of 94°C for 30 sec, 63°C for 30 sec, 72°C for 30 sec and one cycle of 72°C for 10 min. Aliquots of the PCR products were analyzed by electrophoresis in a 1.5% agarose gels. The final products were cloned into pMD 18-T vector (Takara, Japan).

Plasmid extraction, diagnostic digestion and sequencing: White colonies were picked up with a sterile wooden toothpick and were inoculated into tubes with 10 mL of agar containing 100 mg mL^{-1} ampicillin. Tubes were incubated on a shaker at 37°C and 100 rpm for 12-18 h. Plasmids were extracted using a Qiagen Plasmid Purification Mini kit (Qiagen). The 8 μL of plasmid, 8 μL of Dnase free water, 1 μL of both 10 U μL^{-1} EcoR I and 10 U μL^{-1} Hind III and 2 μL of the respective 10X reaction buffer were added to a final volume of 20 μL and incubated for 1 h at 37°C. As a control, 8 μL of uncut plasmid and 12 μL of DNase free water were added to a final volume of 20 μL and incubated for 1 h at 37°C. The vector containing the insert had EcoRI and XbaI restriction enzyme sites. Following diagnostic digestion, digestion products were loaded on a 1% agarose gel with ethidium bromide. Purified plasmids were sequenced (Takara, Japan).

Bioinformatic analysis: The analysis of sequences were performed using the BLAST at the National Center for Biotechnology Information (NCBI) server (http://blast.ncbi.nlm.nih.gov/), the ClustalW Software (http://www.ebi.ac.uk/Tools/clustalw2/index.html) and DNAstar Software.

The computation of various physical and chemical parameters for the protein sequences was used by ProtParam tool (http://web.expasy.org/protparam/). The isoelectric point and molecular weight (pI/Mw) of deduced amino acids sequence was analyzed in compute pI/Mw (http://web.expasy.org/compute_pi/).

The classification Results of Predicted Disulfide Bonds was used by Dlpro (http://scratch.proteomics.ics.uci.edu/). The ScanProsite tool (http://prosite.expasy.org/scanprosite/) was used for detecting PROSITE signature matches in protein sequences. The NetPhos 2.0 Server (http://www.cbs.dtu.dk/services/NetPhos/) was used to predict the phosphorylation sites.

The NetNglyc server predicts N-Glycosylation sites in human proteins using artificial neural networks that examine the sequence context of Asn-Xaa-Ser/Thr sequins (http://www.cbs.dtu.dk/services/NetNGlyc/). The signal peptides analysis was done by SignalP-noTM prediction in SignalP 4.0 Server (http://www.cbs.dtu.dk/services/SignalP/).

The prediction of membrane-spanning regions and orientation was done using the TMpred Server (http://www.ch.embnet.org/software/TMPRED_form.html). The Swiss-Model (http://swissmodel.expasy.org/) was employed to homology modeling of proteins and RasMol Software was used to visualize the PDB files generated by Swiss-Model. The TargetP 1.1 Server (http://www.cbs.dtu.dk/services/TargetP/) was used for predicting Protein Subcellular Localization. The Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway) was used to retrieve the genome sequences.

The Gene Structure Display Server (GSDS) is a web server for drawing gene structure schematic diagram (http://gsds.cbi.pku.edu.cn/chinese.php?input=site/). The search for predicted microRNA targets in mammals was done by TargetScan (http://www.targetscan.org/). The Promoter2.0 (http://www.cbs.dtu.dk/services/ Promoter/) was used to predict transcription start sites of vertebrate promoters in DNA sequences. The TFSEARCH (http://mbs.cbrc.jp/research/db/TFSEARCH.html) was used for searching transcription factor binding sites.

Detection of *FSP27* gene expression: FSP27 mRNA was assayed by real-time quantitative reverse transcriptase Polymerase Chain Reaction (PCR) of RNA samples previously treated with DNAse (DNA free, TaKaRa, Japan). The *18S rRNA* gene was used as an internal control. The primers used were FSP27, 5'-CATCACAGC AGGGCAGTAG-3' (forward) and 5'-CGTAGGAAAGGG AGTAGGC-3' (reverse) and 18S rRNA, 5'-GCGGCTTTG GTGACTCTA-3' (forward) and 5'-CTGCCTCCTTGGAT GTG-3' (reverse). Conditions for cycling were 95°C for 30 sec followed by 40 cycles of denaturation at 95°C for 5 sec, annealing at 60°C for 30 sec for *FSP27* gene and *18S rRNA* gene, extension at 72°C for 30 sec and Melt Curve 65.0-95.0°C increment 0.5°C 15 sec. Samples were assayed in triplicate and each experiment was repeated twice. Changes were expressed in twofold increments as described previously (Zhao *et al*., 2007).

Statistical analysis: Data were analyzed using the general linear model procedure of SAS (Version 8.0; SAS Institute, Inc.). Statistical differences in relative mRNA expression between experimental groups were assessed by Student t-test. All experimental data were expressed as means±Standard Error of the Mean (SEM). Differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Cloning and sequencing of porcine FSP27: Sequencing of the resulting cDNA showed that the complete coding sequence of *FSP27* gene in Wujin and Landrace pigs is 747 bp encoding for 248 amino acids. In Wujin pigs a silent mutation at nucleotides 549 (T to C) was observed. The deduced amino acid sequences of FSP27 showed 100% identity between Wujin and Landrace pigs.

The tissue expression of FSP27 mRNA: The *FSP27* gene was highest expressed in adipose tissues. The ratio of *FSP27-18S rRNA* gene in adipose tissues reached 1.22. The relative expression level of *FSP27* gene was lowest in kidney (ratio was 0.39) (Fig. 1). The expression of FSP27 mRNA in longissimus dorsi muscle was significantly higher in Wujin pigs than in Landrace pigs ($p<0.05$) (Fig. 2). The FSP27 mRNA abundance and IMF content showed a strong linear relationships in Wujin pigs ($R^2 = 0.9161$) (Fig. 3).

Physical and chemical characteristics of the deduced protein: The molecular formula of the deduced amino acid sequence of FSP27 in Wujin pigs was $C_{1242}H_{1997}N_{333}O_{364}$

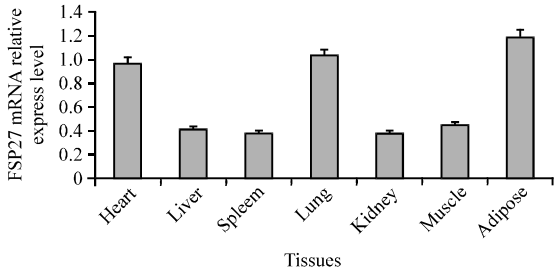

Fig. 1: The tissue expression profile of *FSP27* gene in Wujin pigs. FSP27 mRNA relative expression was revealed by RT-PCR. Total RNA was extracted from heart, liver, spleen, lung, kidney, longissimus dorsi muscle and subcutaneous fatty tissue, respectively. Relative expression level was indicated by the ratio of *FSP27-18S rRNA* gene. Data are expressed as the mean±SE (N = 12)

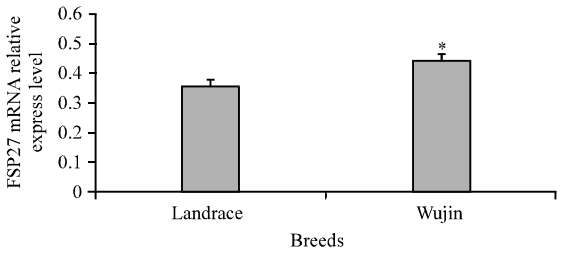

Fig. 2: Relative FSP27 mRNA abundance of longissimus dorsi muscle in Wujin and Landrace pigs. FSP27 mRNA relative expression was revealed by RT-PCR. The total RNA was extracted from longissimus dorsi muscle tissue. Relative expression level was indicated by the ratio of *FSP27-18S rRNA* gene. Data are expressed as the mean±SE (N = 12). Asterisks show significant differences between two pig breeds ($p<0.05$)

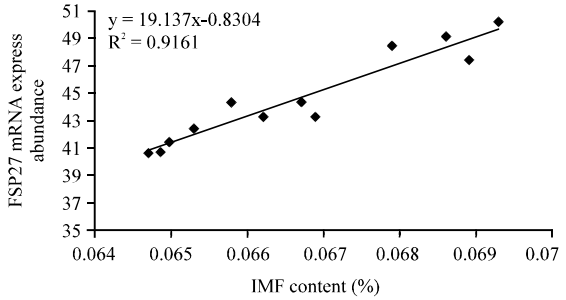

Fig. 3: The relationship between FSP27 mRNA abundance and IMF content of longissimus dorsi muscle in Wujin pigs. The total RNA was extracted from longissimus dorsi muscle tissue. Data are expressed as means±SE of specific mRNA: 18s rRNA for 12 pigs. IMF content of the longissimus dorsi muscles were detected by the Soxhlet Petroleum-Ether Extraction Method

Table 1: The deduced amino acids composition of FSP27 protein in Wujin pigs

Amino acid	Number	Percentage	Amino acid	Number	Percentage
Ala (A)	16	6.5	Leu (L)	34	13.7
Arg (R)	14	5.6	Lys (K)	17	6.9
Asn (N)	4	1.6	Met (M)	9	3.6
Asp (D)	12	4.8	Phe (F)	8	3.2
Cys (C)	6	2.4	Pro (P)	13	5.2
Gln (Q)	14	5.6	Ser (S)	20	8.1
Glu (E)	13	5.2	Thr (T)	17	6.9
Gly (G)	13	5.2	Trp (W)	2	0.8
His (H)	3	1.2	Tyr (Y)	10	4.0
Ile (I)	6	2.4	Val (V)	17	6.9

S_{15}, the total number of atoms was 3951, the total number of negatively charged residues (Asp+Glu) was 25 and the total number of positively charged residues (Arg+Lys) was 31 (Table 1). The FSP27 protein was hydrophilicand and unstable. Isoelectric point and molecular weight (pI and Mw) were approximately 8.95 and 27899.43 Da, respectively. The predicted protein secondary structure revealed that a helix structure account for about 27% of the protein.

The sixty seven helices and 49 strand structures were found in the whole protein. The remaining structures are coils that account for 53% of the protein (Fig. 4). The CIDE-N domain was from 51-128 AA and the SERPIN was from 85-95 AA. The FSP27 protein had 13 predicted phosphorylation sites (Ser: 9, Thr: 3, Tyr: 1). No N-glycosylated sites or potential signal peptide structures were observed. FSP27 protein had two transmembrane helices structures. Swiss-Model was employed to homology modeling of proteins. One model of porcine FSP27 from Gallus and Quail was selected (E-value: 1.10e-34, QMEAN Z-Score: -0.42, QMEAN4: 0.752). FSP27 modeled residue for Gallus range from 47-133 which has 49.43% sequence identity with template

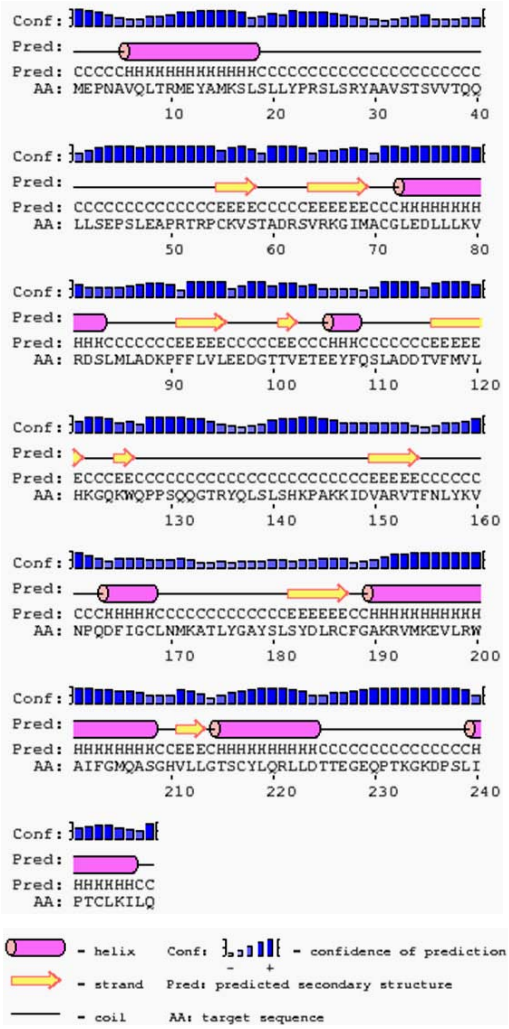

Fig. 4: Secondary structure of *FSP27* gene-coding proteins. The secondary structure of *FSP27* gene-coding proteins had 67 helices, 49 strands and 132 coils

Table 2: The length of transcription region of FSP27 in human, pig, mouse and rat

Species	Number and length (bp) of exon	Length (bp) of 5'-UTR	Length (bp) of CDS	Length (bp) of 3'-UTR
Human	6 (115, 78, 154, 159, 188, 583)	140	717	420
Mouse	6 (47, 78, 154, 159, 191, 1091)	72	720	928
Pig	6 (33, 78, 154, 159, 188, 682)	28	747	519
Rat	6 (23, 78, 154, 159, 191, 1024)	48	717	864

sequence (Fig. 5). The subcellular localization result showed that FSP27 protein in Wujin pigs was localized in the mitochondria.

Structure prediction analysis of non-coding regions of FSP27/CIDEC: Genome Browser was used to retrieve the FSP27 genome sequences of pig, human, mouse and rat (Table 2).

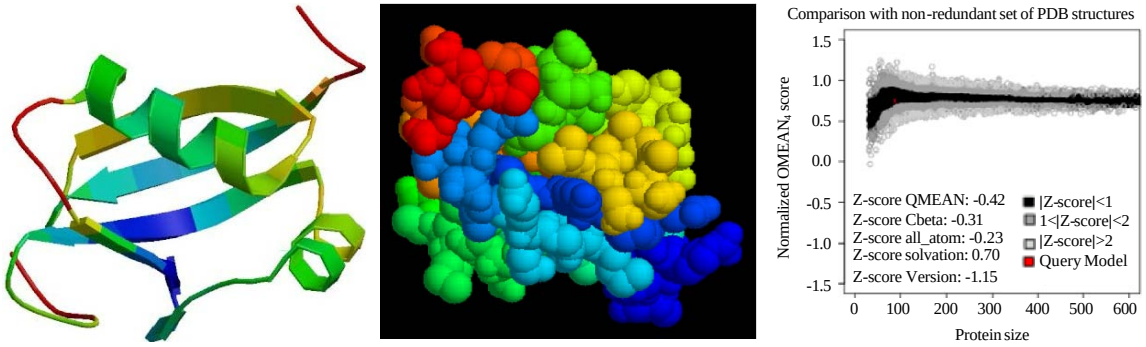

Fig. 5: The predicted cartoon model, molecular surface model of FSP27 protein and its model quality plot. Regarding to local model quality, estimated per-residue inaccuracy visualized using a color gradient from blue (more reliable regions) to red (potentially unreliable regions)

Table 3: The potential targets for miRNA's in the 3-'UTR region of the *FSP27* gene in Wujin pigs

Names	Pairing region	Length
Position 102-109 of CIDEC 3' UTR	5' GUAGAGCCGCGAACC--UCCCCACA	8 mer
	||| |||||	
hsa-miR-491-5p	3' GGAGUACCUUCCCAAGGGGUGA	
Position 111-118 of CIDEC 3' UTR	5' CGAACCUCCCCACACCUCCCUCA	8 mer
	|||||	
hsa-miR-4510	3' UUGGUAUGUAGGAUGAGGGAGU	
Position 111-118 of CIDEC 3' UTR	5' CGAACCUCCCCACACCUCCCUCA	8 mer
	|||||	
hsa-miR-4419a	3' ACGUCAGAGGAGGGAGU	
Position 277-283 of CIDEC 3' UTR	5' CCAUUGGCAUGAAGUCUGCCCCU	7 mer
	|||||	
hsa-miR-486-3p	3' UAGGACAUGACUCGACGGGGC	
Position 278-284 of CIDEC 3' UTR	5' CAUUGGCAUGAAGUCUGCCCCUU	7 mer
	|||||	
hsa-miR-4688	3' GGGUCCAGGAGACGACGGGGAU	

The 3'-UTR sequence of FSP27 showed that a 3' end polyadenylation signal (AATAAA) was located at the approximately 16 bp upstream of the poly (A) tail. A search for predicted microRNA targets in mammals was done by TargetScan Software (Table 3). Human data were used for this analysis since porcine data were not yet in the database.

Predicted transcription start sites of vertebrate promoters results showed that the promoter region extends likely to -2000 bp at the upstream (5' end) of *FSP27* gene. The promoter region of *FSP27* gene showed transcription factor binding sites for example for NF-κ-β, AML-1a and SRY. In addition, researchers found that the TATA box was located at -1610 bp upstream of transcription start site (Fig. 6).

FSP27, a fat-specific protein of 27 kDa in mouse is also known as Cell death-inducing DFFA-like effector c(CIDEC) protein. CIDEC, CIDEA and CIDEB are three members of CIDE pro-apoptotic proteins family which harbor an N-terminal domain and a C-terminal domain (Inohara *et al*., 1998). CIDE proteins have high affinity to DNA fragmentation factors and play important roles in apoptosis and DNA fragmentation (Xu *et al*., 2012). FSP27 colocalizes with perilipin at the amino acid level: the 2-29 AA is a short N-terminal sequence with homology to adipophilin, the 46-77 AA is a segment with homology to a region of perilipin thought to protect LD from lipases (Puri *et al*., 2008). Further, depletion of FSP27 in cultured adipocytes caused LD fragmentation and an increase in lipolysis whereas its expression in non-adipose cells increases LD size and TG levels (Traini and Jessup, 2009; Puri *et al*., 2007; Liu *et al*., 2009). Serine protease inhibitor (Serpin) plays a key role during the physiological processes such as apoptosis, blood congealing, immunoreactions, plasmin fusion, inflammation and tumor suppressor (Irving *et al*., 2002; Meyer-Hoffert *et al*., 2010). Therefore, FSP27 of Wujin pigs could also regulate lipolysis and apoptosis in adipocytes.

Protein subcellular localization plays an important role in the functional divergence and retention of duplicate genes, the subcellular localization of the CIDEA and CIDEB was determined to be in the mitochondria (Hibbetts *et al*., 1999; Chen *et al*., 2000). Recent studies revealed that FSP27 localizes to LD in 3T3L1 adipocytes (Puri *et al*., 2007). However, murine with deficiencies in CIDEC/Fsp27 display lean phenotypes, higher energy expenditure and insulin resistance, suggesting that

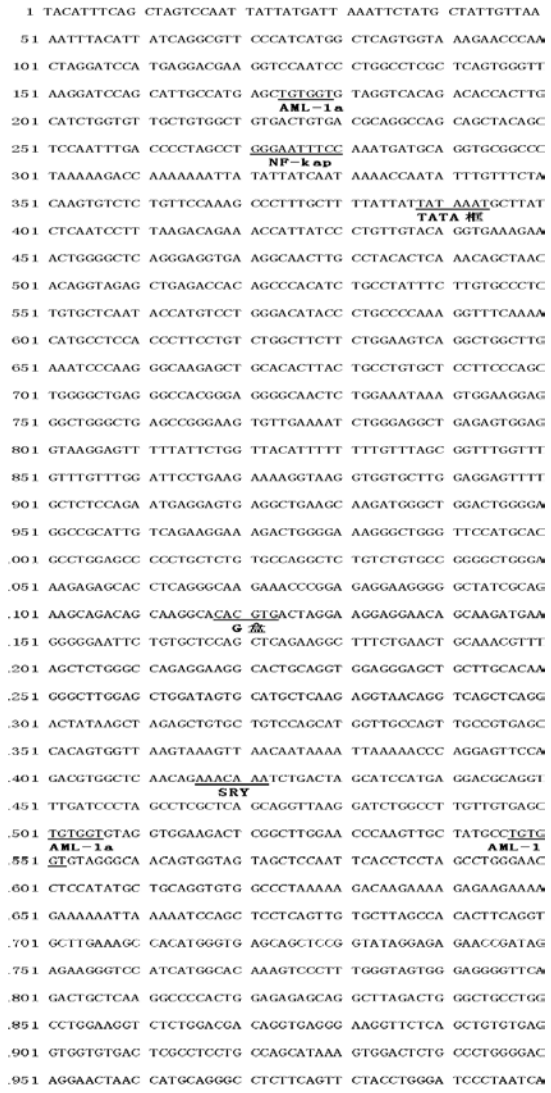

Fig. 6: The regulatory sites in promoter region of *FSP27* gene in Wujin pigs The promoter region of *FSP27* gene had AML-1a, NF-κ and SRY transcription factor binding sites. The TATA box was located at -1610 bp upstream of the transcription start site

CIDEC/Fsp27 is associated with energy expenditure, possibly via mitochondrial function (Nishino *et al*., 2008). Further, Puri and Czech (2008) speculated that FSP27 is a possible intermediary effect to increased intracellular fatty acid metabolism via mitochondrial mechanisms by two candidate signal pathways. The first signal pathway includes nuclear receptors such as the PPAR protein family, known to be responsive to fatty acids and their derivatives. PPARγ powerfully promotes adipogenesis, a process also associated with increased mitochondrial biogenesis. The other pathway could be the protein kinase AMPK which is a key regulator of fatty acid oxidation in response to increase intracellular AMP levels. Therefore, FSP27 might promote adipogenesis and fatty acid oxidation by PPARγ and AMPK signal pathways.

The analysis of genomic structure such as exon length and intron phase patterns showed that the ancestral CIDE-N domain had undergone different intron insertions to various positions in the domain among invertebrates, the genomic structure of CIDE family in vertebrates is stable with conserved intron phase (Wu *et al*., 2008). Jambunathan *et al*. (2011) revealed that amino acids 120-210 are necessary and sufficient for both clustering and fusion of LDs to form larger droplets. The *FSP27* gene has 6 conservative exons of which 3 are identical in length to other species (78, 154, 159). The 3 conserved exons (154, 159, 188) of FSP27 in pig could to be necessary and sufficient for both clustering and fusion of LDs to form enlarged droplets. The different length of transcription region of FSP27 might relate to an intron of CIDE-N domain insertions to various positions as compared to other species.

The CIDEC transcript is inversely regulated by Tumor Necrosis Factor (TNF)-α and insulin which is consistent with an antilipolytic function (Kim *et al*., 2008a). Further, study of the putative transcription factor binding sites in the 5'-upstream region of mouse FSP27 showed PPAR, Hepatocyte Nuclear Factor-3 (HNF-3), GATA-binding protein 3 (GATA3), Sterol Regulatory Element-Binding Protein-1 (SREBP-1), CAMP Response Element-Binding Protein (CREBP) and C/EBP (Matsusue, 2009). This mean that NF-κ, AML-1a and SRY could target regulate porcine *FSP27* gene. The promoter region is likely to extend beyond -2000 bp upstream.

FSP27 is predominantly expressed in both Brown Adipose Tissue (BAT) and White Adipose Tissue (WAT) (Li *et al*., 2010a; Karbowska and Kochan, 2012). And FSP27 is enriched at the LD-LD Contact Sites (LDCSs) and promote lipid exchange and lipid transfer between LDs that are in contact, resulting in the final growth and enlargement of LDs in adipocytes (Gong *et al*., 2011; Li *et al*., 2010b; Karbowska and Kochan, 2012). The biological role and mapping localization suggested that FSP27 could be a promising functional and positional candidate gene for LDs formation and lipid metabolism (Magnusson *et al*., 2008; Ito *et al*., 2010). In the study, the highest expression of FSP27 in Wujin pigs is in adipose tissue which was consistent with previous research in humans (Magnusson *et al*., 2008), swine (Li *et al*., 2009), bovine (Wang *et al*., 2013) and rodents (Kim *et al*., 2008a).

Besides, mouse FSP27 is highly and specifically expressed in BAT and WAT it was expressed at lower levels in the normal mouse liver (Kim *et al*., 2008a; Zhou *et al*., 2003; Matsusue *et al*., 2008; Toh *et al*., 2008). This data indicates that the *FSP27* gene is heavily involved in lipogenesis. Furthermore, research also found that *FSP27* gene was expressed at high levels in lungs of ob/ob mouse (Matsusue *et al*., 2008), bovine (Wang *et al*., 2013) and swine (Li *et al*., 2009) which was consistent with existing research. Interesting, previous study showed weak expression of FSP27 in heart of porcine (Li *et al*., 2009) which is inconsistent with this study. FSP27 protein showed eight transmembrane helices. This feature could add to the mechanism of the fat deposition as it may indicated a transmembrane cellular importation function for FSP27. Although, the function of the *FSP27* gene in pig is not clear yet the differential expression of this gene in tissues suggested that it might posses a unique function in Wujin pigs.

Previous reports indicated that IMF content in Wujin pigs was significantly higher than in Landrace pigs and the average adipocyte diameter in Wujin pigs was greater than Landrace pigs (Zhao *et al*., 2009). Moreover, FSP27 overexpression in mice could promote fat accumulation and induces the formation of large lipid droplets (Keller *et al*., 2008). Inversely, its inhibition or knockout led to the formation of a large number of small lipid droplet, increased lipolysis and reduced fat deposition. FSP27 plays a unique role in LD dynamics. Accumulating evidence indicates that FSP27 plays a role in TG accumulation and LD size in adipocytes and liver (Matsusue, 2009; Kim *et al*., 2008b). These studies suggest that researchers can change the form of aggregation of lipid droplets in fat cells by affecting the expression of FSP27.

The present study showed that FSP27mRNA abundance of longissimus dorsi muscle in Wujin pigs was higher compared with Landrace pigs. And FSP27 mRNA abundance and IMF content showed a strong linear relationships in Wujin pigs ($R^2 = 0.9161$). Therefore, these results suggested that the different expression of FSP27 mRNA in longissimus dorsi muscle between Wujin and Landrace pigs may result in the variation of IMF deposition in the two breeds.

CONCLUSION

In this study researchers first isolated the *FSP27* gene in longissimus dorsi muscle of Wujin pigs and performed bio-informational analysis. The different expression levels of the FSP27 mRNA in longissimus dorsi muscle of pigs may relate to the variation of the IMF deposition. Moreover, research of *FSP27* gene regulation lipid anabolism in porcine intramuscular adipocytes will be necessary in future. The study is beneficial to molecular breeding practice of *FSP27* gene which was a candidate gene in porcine intramuscular fat deposition.

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