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A Novel Pig Gene, PFKFB1, Differentially Expressed in the Muscle Tissues from Wujin Pigs and Large White Pigs

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Abstract: The mRNA differential display technique was performed to investigate gene expression differences in the longissimus dorsi muscle from Wujin and Large white pigs. A fragment of one differentially expressed gene was isolated and sequenced. A complete cDNA sequence of the gene was obtained using the Rapid Amplification of Cdna Ends (RACE) method. The open reading frame of this gene encodes a protein of 428 amino acids which is homologous with the 6-Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 1 (PFKFB1) of 6 species: cattle (97%), human (97%), horse (96%), rhesus monkey (96%), rat (94%) and mouse (94%). This newly identified gene was respectively defined as the swine PFKFB1 gene and had been assigned GeneID: 100233197. The phylogenetic tree analysis revealed that the swine PFKFB1 gene has a closer genetic relationship with the PFKFB1 gene of cattle. The tissue expression analysis indicated that the swine PFKFB1 gene has a broad tissue distribution. The experiment is the first to establish the primary foundation for further research on the swine PFKFB1 gene.

Key words: Pig, PFKFB1, muscle tissue, mRNA differential display, gene, China

INTRODUCTION

The mRNA differential display first described by Liang and Pardee (1992) remains an efficient tool for comparative profiling of gene expression under different experimental conditions. It has statistically been shown that 80-120 primer combinations would be sufficient to cover all the transcript populations in the cell (Liang *et al*., 1993). This assay possesses the following advantages: it is based on well established methods, >2 samples can be compared simultaneously and only a small amount of starting material is needed (Yamazaki and Saito, 2002).

Chinese indigenous pig breeds such as Wujin, Meishan, Erhualian and Tongcheng often have conspicuous flaws such as superabundant fat and too low lean meat rate while exotic pig breeds such as Large white, Landrace and Duroc always have lower fat and higher lean meat rates. Therefore, Chinese indigenous pigs are always named fat-type pigs while exotic pigs are always named the lean-type (Pan *et al*., 2003). Given that phenotypic variances are mainly determined by the genetic differences, the identification of differentially expressed genes between Chinese indigenous and exotic pig breeds can give considerable promises for breeding.

The present study was carried out with the mRNA differential display technique to identify the differentially expressed genes in the muscle tissues from one Chinese indigenous breed (Wujin) and one exotic pig breed (Large white). We provide here the results on the identification of previously unrecognised porcine gene, PFKFB1 which is differentially expressed in Large white versus Wujin skeletal muscle tissues.

MATERIALS AND METHODS

Sample collection: The longissimus dorsi muscle samples were collected from 120 days old Large white (5 males and 5 females) and Wujin (5 males and 5 females) pigs for mRNA differential display and semi-quantitative Reverse-Transcription Polymerase Chain Reaction (RT-PCR) analyses.

The tissues including spleen, ovary, heart, small intestine, liver, lung, kidney, muscle and fat were collected from one adult Wujin x Large white cross pig for the later tissue expression profile analysis. Tissues were immediately frozen in liquid nitrogen and stored at -80°C. The total RNA was extracted from tissues using the total RNA extraction kit (Gibco, Grand Island, NY, USA) in accordance with the manufacturer's recommendations. Before the first-strand complementary DNA (cDNA) synthesis, DNase I treatment of the total RNA was done.

Differential display: The differential display PCR amplification of each reverse transcription product was carried out with 10 arbitrary and nine oligo (dT) primers as described by Liu *et al*. (2004, 2005). The PCR products

were then separated on the 8% non-denaturing polyacrylamide gel and displayed using the silver stain (Liu *et al*., 2004, 2005).

Semi-quantitative RT-PCR: Semi-quantitative RT-PCR was performed as described by Liu (2009) and Liu and Xiong (2009a, b). To avoid the influence of cDNA concentration on semi-quantitative RT-PCR, we repeated PCR amplifications using 100-500 ng cDNA as template. We selected the housekeeping gene GAPDH as the internal control. The control gene primers used were: 5'-ACCACAGTCCATGCCATCAC-3' (forward primer 1) and 5'- TCCACCACCCTGTTGCTGT -3' (reverse primer 1). The 420 bp PCR product was verified by sequencing. The following primers were used to perform the RT-PCR for identification and tissue expression profile analysis of the swine PFKFB1 gene: 5'-CAGTCCCTGCC CACTACT-3' (forward primer 2) and 5'-AGCTCCTTGGTT GTAGCTAG-3' (reverse primer 2).

The PCR product was 208-bp in length (verified by sequencing). The 25 μL reaction system contains 2 μL cDNA (100-500 ng), 5 pmoles each oligonucleotide primer (forward primer 1 and 2, reverse primer 1 and 2), 2.5 μL 2 mmol L^{-1} mixed dNTPs, 2.5 μL 10× Taq DNA polymerase buffer, 2.5 μL 25 mmol L^{-1} $MgCl_2$ and 2 units of Taq DNA polymerase. The PCR program initially started with a 94°C denaturation for 4 min followed by 25 cycles of 94, 60 and 72°C/50 sec then 72°C extension for 10 min, finally 4°C to terminate the reaction. PCR products were analysed in the linear range of amplification by agarose gel electrophoresis and intensity of bands was estimated using Glyco BandScan software (PROZYME®, San Leandro, CA, USA). The ratio of PFKFB1 to GAPDH was calculated using Excel program. Difference significance of ratios of PFKFB1 to GAPDH was analyzed with the Least square method (GLM procedure, SAS version 8.0).

Rapid amplification of cDNA ends (5'- and 3'-RACE): 5'- and 3'-RACE were performed as the instructions of BD SMART™ RACE cDNA amplification kit (BD science, USA). The Gene-Specific Primers (GSPs) were: 5'-RACE GSP: 5'-GAAGCAGGTCTCTTCCTGTGAGGCC-3',3'-RACE GSP:5'-CTCTGGACTTGGCCTCACAGGAAGA-3'.RACE touchdown PCRs were carried out with 5 cycles of 94°C/30 sec and 72°C/3 min followed by 5 cycles of 94°C/30 sec, 70°C/30 sec and 72°C/3 min, finally with 30 cycles of 94°C/30 sec, 67°C/30 sec, 72°C/3 min to terminate reaction.

The RACE PCR products were then cloned into pMD18-T vector (TaKaRa, Dalian, China) and sequenced bidirectionally with the commercial fluorometric method (Shenggong, Shanghai, China). At least five independent clones were sequenced for each PCR product.

Sequence analysis: The cDNA sequence prediction was conducted using GenScan software. Protein sequence prediction and analysis were performed using the conserved domain architecture retrieval tool of BLAST at the National Centre for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov/BLAST) and Clustal W software (http://www.ebi.ac.uk/clustalw).

RESULTS AND DISCUSSION

mRNA differential display: From the mRNA differential display, one band, nominated as the band 162, later identified as a fragment of the PFKFB1 gene was found to be predominantly expressed in the longissimus dorsi muscle of Large white pigs while it was barely visible in the band pattern of the longissimus dorsi of Wujin pigs (Fig. 1).

Semi-quantitative RT-PCR: The differentially expressed band was recovered from gel and used as the template for the re-amplification which was performed with the corresponding oligo (dT) and arbitrary primers used in the mRNA differential display assay. The resulting PCR product was 313 bp that was consistent with that of the differential display (Fig. 1). The purified PCR product was then cloned into the pMD18-T vector and the recombinant plasmid was sequenced. Semi-quantitative RT-PCR was conducted and the results (Fig. 2) indicated

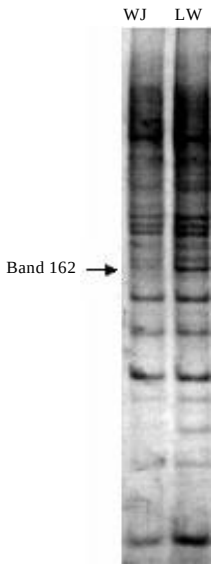

Fig. 1: Representative band pattern on mRNA differential display analysis showing upregulated band 162 (arrow, about 300 bp) in Large White (LW) vs Wujin (WJ) longissimus dorsi muscles

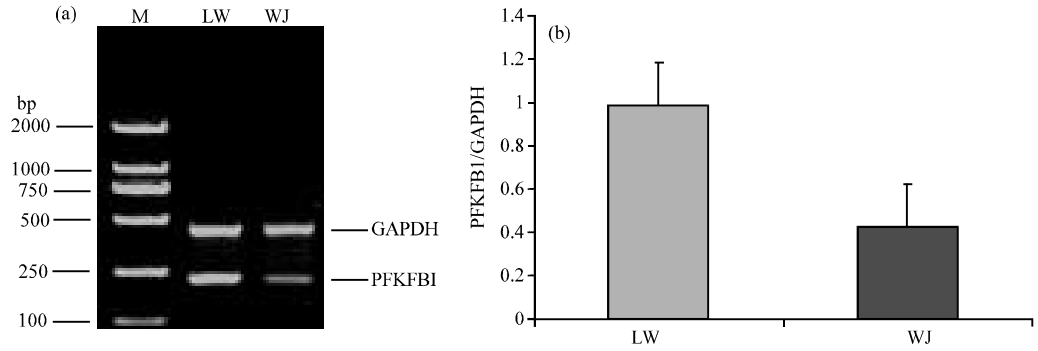

Fig. 2: (a) Representative and (b) calculated PFKFB1 mRNA expression levels in Large White (LW) vs Wujin (WJ) longissimus dorsi muscle samples (RT-PCR, n = 5)

ACCCAAGCCCCCACTGGTAGATACAGGAGCAGAGGCAGGCAAGGCAGGCAGGACTAGGAGA
GAGTAGAAGGGCTGGAGACAGGCTGCCAGAGGGCTCAGAACCCCAGCGCACCTCCCTGTCCC
AACTCTGTCAACCTCCTGCTGTGGCCACTGCAACAGAAGAGACAGCTAATAAGACAGGAAGT
GAGGCCTGGTACCTTGTGGACAGTGGTGTCATTAGCTGCGACGCCTAAGATGACTCAAGAGA
TGGGAGAGCTCACCCAAACC
MTQEMGELTQT
AGATTGCAGAAGATCTGGATTCCACATAGCAGCGGCAACAGTAGGCTG
CAACGGAGGAGGGGCTCA
RLQKIWIPHSSGNSRLQRRRGS
TCCATACCCCAGTTTACAAATTCCCCCACGATGGTGATTATGGTGGGTT
TACCAGCTCGAGGAAAG
SIPQFTNSPTMVIMVGLPARGK
ACCTACATCTCCACGAAGCTCACACGTTATCTCAATTGGATAGGAACAC
CAACTAAAGTGTTTAAT
TYISTKLTRYLNWIGTPTKVFN
TTAGGCCAGTATCGACGAGAGGCAGTGAGCTACAAGAACTACGAATTC
TTTCTCCCAGACAACATG
LGQYRREAVSYKNYEFFLPDNM
GAGGCCCTACTTATAAGGAAGCAGTGTGCCCTGGCAGCTCTGAAAGAT
GTCCATAACTATCTTAGC
EALLIRKQCALAALKDVHNYLS
CATGAGGAAGGTCACGTTGCGGTTTTTGATGCCACCAATACTACCAGAG
AAAGACGGTCTTTGATT
HEEGHVAVFDATNTTRERRSLI
CTACAGTTTGCTAAAGAACACGGTTATAAGGTCTTTTTCATTGAGTCCA
TTTGTAATGACCCCGAC
LQFAKEHGYKVFFIESICNDPD
GTCATTGCAGAAAACATCAGGCAAGTGAAGCTTGGCAGCCCTGATTAT
ATAGACTGTGACCGTGAA
VIAENIRQVKLGSPDYIDCDRE
AAGGTTCTAGAAGACTTTCTAAAAAGAATCCAGTGCTATGAGGTCAAC
TACCAACCTTTGGATGAT
KVLEDFLKRIQCYEVNYQPLDD
GAACTGGACAGCCACTTGTCCTACATCAAGATCTTCGACGTGGGCACAC
GCTACATGGTGAACCGC

Fig. 3: Continued

ELDSHLSYIKIFDVGTRYMVNR
GTGCAGGACCACATCCAGAGCCGCACAGTCTACTATCTCATGAACATCCATGTCACACCCCGCTCC
VQDHIQSRTVYYLMNIHVTPRS
ATCTACCTATGCCGGCACGGTGAGAGTGAACTCAACCTCAGAGGCCGCATCGGAGGTGACTCTGGC
IYLCRHGESELNLRGRIGGDSG
CTCTCACCTCGGGGCAAGCAGTATGCCTATGCCTTGGCCGACTTCATTAAGTCCCAGGCCATCAGC
LSPRGKQYAYALADFIKSQAIS
TCCCTGAAGGTGTGGACCAGCCACATGAAGAGGACTATCCAGACAGCTGAAGCCCTGGATGTCCCC
SLKVWTSHMKRTIQTAEALDVP
TATGAGCAGTGGAAGGCCCTGAATGAGATTGATGCGGGTGTCTGTGAAGAAATGACATATGAGGAA
YEQWKALNEIDAGVCEEMTYEE
ATCCAAGAGCACTATCCCGAAGAATTTGCCCTACGAGACCAAGATAAATATCGCTACCGCTATCCC
IQEHYPEEFALRDQDKYRYRYP
AAGGGAGAGTCCTATGAAGATCTGGTCCAGCGTCTGGAGCCAGTTATAATGGAGCTAGAACGTCAG
KGESYEDLVQRLEPVIMELERQ
GAAAATGTATTAGTGATTTGCCACCAGGCTGTCATGCGGTGCCTCCTGGCCTACTTCCTGGACAAG
ENVLVICHQAVMRCLLAYFLDK
AGCTCAGATGAGCTGCCATATCTCAAGTGCCCTCTGCACACAGTGCTCAAACTTACGCCTGTGGCT
SSDELPYLKCPLHTVLKLTPVA
TATGGCTGCAAAGTGGAGTCGATCTACCTGAATGTGGAGGCTGTCAACACACACCGGGAGAAGCCT
YGCKVESIYLNVEAVNTHREKP
GAGAATGTAGACATCACCCGAGAACCTGAGGAAGCTCTGGACACAGTCCCTGCCCACTACTGAGCAC
ENVDITREPEEALDTVPAHY?
TTTCTAAGACATCAAACTTCCTCTGTCCTAGCTCTCTTCCAACTTTAGGAGGTGA
CGTCATTGTTCTCCTACCCTGAGAATACTCTGGACTTGGCCTCACAGGAAGAGAC
CTGCTTCCAGTGAAGAAACTCTCATCAGCTCTGAAACAAGTCTTGACGTCTAGCT
ACAACCAAGGAGCTATCTAGCTCAGGAAGAAACTTTTTCTTTCTTAATTCCTATT
CCCTAGTCAATAAAGACTTCTGTTACTGACCAAAAAAAAAAA

Fig. 3: The cDNA and amino acid sequence of the swine gene containing band 162 (GenBank accession number: FJ436397). ATG, start codon; TGA, stop codon (* -stop codon)

that the band 162 (PFKFB1) is predominantly expressed in the longissimus dorsi muscle of Large white pigs. Through, 5'-RACE, one PCR product of 1771 bp was obtained. The 3'-RACE product was 180 bp. These products were then cloned to t-vector and sequenced. The alignment of these 35 bp overlapping sequences yielded a 1916 bp cDNA sequence (Fig. 3). The nucleotide analysis using the BLAST software at NCBI server (http://www.ncbi.nlm.nih.gov/BLAST), revealed that a 1916 bp cDNA sequence was not homologous to any of the known porcine genes and it was then deposited into the GenBank database (Accession number: FJ436397). The sequence prediction was carried out using the GenScan software.

An Open Reading Frame (ORF) encoding 428 amino acids was found in the 1916-bp cDNA sequence. A probability of exon was 0.777, poly-A signal was from 1883-1888 bp (consensus: AATAAA). The complete cDNA sequence of this gene and the encoded amino acids are shown in Fig. 3.

Further BLAST analysis revealed that the protein sequence is characterized by a high homology with that of the 6-Phosphofructo-2-Kinase/ Fructose-2,6-Biphosphatase 1 (PFKFB1) of cattle (Accession number: NP_776997; 97%), human (Accession number: NP_002616; 97%), horse (Accession number: XP_001494106; 96%), rhesus monkey (Accession number: XP_001091907; 96%), rat (accession number: NP_036753; 94%), mouse (accession number: P70266; 94%) (Fig. 4).

On the basis of results obtained in the experiments we assume that this gene can be defined as the swine PFKFB1 gene. Based on the results of the alignment of six known PFKFB1 proteins, a phylogenetic tree was constructed using the Clustal W software (Fig. 5).

The swine PFKFB1 gene has a closer genetic relationship with the cattle PFKFB1 gene than with human, horse, rhesus monkey, rat and mouse ones.

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Pig              MTQEMGELTQTRLQKIWIPHSSGNSRLQRRRGSSIPQFTNSPTMVIMVGLPARGKTYIST
Cattle           MSQEMGELTQTRLQKIWIPHNNGNSRLQRRRGSSIPQFTNSPTMVIMVGLPARGKTYIST
Horse            MSQEMGELTQTRLQKIWIPHSSGSSGLQRRRGSSIRQFTNSPTMVIMVGLPARGKTYIST
Human            MSPEMGELTQTRLQKIWIPHSSGSSRLQRRRGSSIPQFTNSPTMVIMVGLPARGKTYIST
Rhesus monkey    MSPEMGELTQTRLQKIWIPHSSGSSRLHRRRGSSIPQFTNSPTMVIMVGLPARGKTYIST
Rat              MSREMGELTQTRLQKIWIPHSSSSSVLQRRRGSSIPQFTNSPTMVIMVGLPARGKTYIST
Mouse            MSREMGELTQTRLQKIWIPHSSSSSLLQRRRGSSIPQFTNSPTMVIMVGLPARGKTYIST
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Pig              KLTRYLNWIGTPTKVFNLGQYRREAVSYKNYEFFLPDNMEALLIRKQCALAALKDVHNYL
Cattle           KLTRYLNWIGTPTKVFNLGQYRREAVSYKNYEFFLPDNMEALLIRKQCALAALKDVHSYL
Horse            KLTRYLNWIGTPTKVFNLGQYRREAVSYKNYEFFLPDNMEALLIRKQCALAALKDVHDYL
Human            KLTRYLNWIGTPTKVFNLGQYRREAVSYKNYEFFLPDNMEALQIRKQCALAALKDVHNYL
Rhesus monkey    KLTRYLNWIGTPTKVFNLGQYRREAVSYKNYEFFLPDNMEALQIRKQCALAALKDVHNYL
Rat              KLTRYLNWIGTPTKVFNLGQYRREAVSYRNYEFFRPDNTEAQLIRKQCALAALKDVHKYL
Mouse            KLTRYLNWIGTPTKVFNLGQYRREAVSYRNYEFFRPDNMEAQLIRKQCALAALKDVHKYL
                 ****************************:***** *** **  **************.**

Pig              SHEEGHVAVFDATNTTRERRSLILQFAKEHGYKVFFIESICNDPDVIAENIRQVKLGSPD
Cattle           SHEEGRVAVFDATNTTRERRSLILQFAKEHGYKVFFIESICNDPDVIAENIRQVKLGSPD
Horse            SHEEGHVAVFDATNTTRERRSLILQFAKEHGYKVFFIESICNDPGIIAENIRQVKLGSPD
Human            SHEEGHVAVFDATNTTRERRSLILQFAKEHGYKVFFIESICNDPGIIAENIRQVKLGSPD
Rhesus monkey    SREEGHVAVFDATNTTRERRSLILQFAKEHGYKVFFIESICNDPGVIAENIRQVKLGSPD
Rat              SREEGHVAVFDATNTTRERRSLILQFAKEHGYKVFFIESICNDPEIIAENIKQVKLGSPD
Mouse            SREEGHVAVFDATNTTRERRSLILQFAKEHGYKVFFIESICNDPDIIAENIKQVKLGSPD
                 *:***:************************************** :*****:********

Pig              YIDCDREKVLEDFLKRIQCYEVNYQPLDDELDSHLSYIKIFDVGTRYMVNRVQDHIQSRT
Cattle           YIDCDREKVLEDFLKRIECYEVNYQPLDDELDSHLSYIKIFDVGTRYMVNRVQDHIQSRT
Horse            YIDCDREKVLEDFLKRIECYKVNYQPLDDELDSHLSYIKIFDVGTRYMVNRVQDHIQSRT
Human            YIDCDREKVLEDFLKRIECYEVNYQPLDEELDSHLSYIKIFDVGTRYMVNRVQDHIQSRT
Rhesus monkey    YVDCDREKVLEDFLKRIECYEVNYQPLDEELDSHLSYIKIFDVGTRYMVNRVQGHIQSRT
Rat              YIDCDQEKVLEDFLKRIECYEINYQPLDEELDSHLSYIKIFDVGTRYMVNRVQDHVQSRT
Mouse            YIDCDQEKVLEDFLKRIECYEINYQPLDEELDSHLSYIKIFDVGTRYMVNRVQDHVQSRT
                 *:***:***********:**::******:************************.*:****

Pig              VYYLMNIHVTPRSIYLCRHGESELNLRGRIGGDSGLSPRGKQYAYALADFIKSQAISSLK
Cattle           VYYLMNIHVTPRSIYLCRHGESELNLRGRIGGDSGLSARGKQYAYALANFIQSQGISSLK
Horse            VYYLMNIHVTPRSIYLCRHGESELNLRGRIGGDSGLSARGKQYAYALANFIQSQGISSLK
Human            VYYLMNIHVTPRSIYLCRHGESELNIRGRIGGDSGLSVRGKQYAYALANFIQSQGISSLK
Rhesus monkey    VYYLMNIHVTPRSIYLCRHGESDLNLRGRIGGDSGLSVRGKQYAYALANFIQSQGISSLK
Rat              AYYLMNIHVTPRSIYLCRHGESELNLRGRIGGDSGLSARGKQYAYALANFIRSQGISSLK
Mouse            AYYLMNIHVTPRSIYLCRHGESELNLRGRIGGDSGLSARGKQYAYALANFIRSQSISSLK
                 .*********************:**:*********** **********:**:**.*****

Pig              VWTSHMKRTIQTAEALDVPYEQWKALNEIDAGVCEEMTYEEIQEHYPEEFALRDQDKYRY
Cattle           VGTSHMKRTIQTAEALGLPYEQWKALNEIDAGVCEEMTYEEIQEHYPEEFALRDQDKYRY
Horse            VWTSHMKRTIQTAEALGVPYEQWKALNEIDAGVCEEMTYEEIQEHYPEEFALRDQDKYRY
Human            VWTSHMKRTIQTAEALGVPYEQWKALNEIDAGVCEEMTYEEIQEHYPEEFALRDQDKYRY
Rhesus monkey    VWTSHMKRTIQTAEALGVPYEQWKALNEIDAGVCEEMTYEEIQEHYPEEFALRDQDKYRY
Rat              VWTSHMKRTIQTAEALGVPYEQWKALNEIDAGVCEEMTYEEIQEHYPEEFALRDQDKYRY
Mouse            VWTSHMKRTIQTAEALGVPYEQWKALNEIDAGVCEEMTYEEIQEHYPEEFALRDQDKYRY
                 * **************.:******************************************

Pig              RYPKGESYEDLVQRLEPVIMELERQENVLVICHQAVMRCLLAYFLDKSSDELPYLKCPLH
Cattle           RYPKGESYEDLVQRLEPVIMELERQENVLVICHQAVMRCLLAYFLDKSSDELPYLKCPLH
Horse            RYPKGESYEDLVQRLEPVIMELERQENVLVICHQAVMRCLLAYFLDKSSDELPYLKCPLH
Human            RYPKGESYEDLVQRLEPVIMELERQENVLVICHQAVMRCLLAYFLDKSSDELPYLKCPLH
Rhesus monkey    RYPKGESYEDLVQRLEPVIMELERQENVLVICHQAVMRCLLAYFLDKSSDELPYLKCPLH
Rat              RYPKGESYEDLVQRLEPVIMELERQENVLVICHQAVMRCLLAYFLDKSSDELPYLKCPLH
Mouse            RYPKGESYEDLVQRLEPVIMELERQENVLVICHQAVMRCLLAYFLDKSSDELPYLKCPLH
                 ************************************************************

Pig              TVLKLTPVAYGCKVESIYLNVEAVNTHREKPENVDITREPEEALDTVPAHY
Cattle           TVLKLTPVAYGCKVESIYLNVEAVNTHREKPENVDITREPEEALDTVPAHY
Horse            TVLKLTPVAYGCKVESIYLNVEAVNTHREKPENVDITREPEEALDTVPDHY
Human            TVLKLTPVAYGCKVESIYLNVEAVNTHREKPENVDITREPEEALDTVPAHY
Rhesus monkey    TVLKLTPVAYGCKVESIYLNVEAVNTHREKPENVDITREPEEALDTVPAHY
Rat              TVLKLTPVAYGCRVESIYLNVEAVNTHRDKPENVDITREAEEALDTVPAHY
Mouse            TVLKLTPVAYGCRVESIYLNVEAVNTHRDKPENVDITREPEEALDTVPAHY
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Fig. 4: The alignment of PFKFB1 proteins

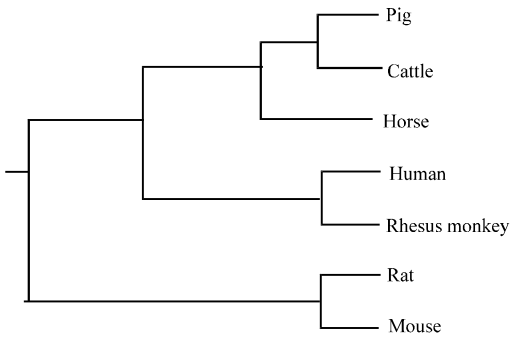

Fig. 5: The phylogenetic tree for the PFKFBI genes

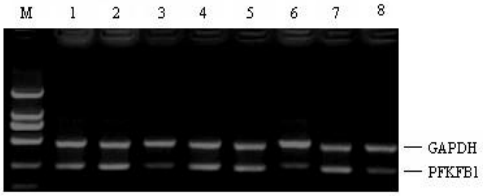

Fig. 6: RT-PCR profiling of the swine PFKFB1 gene expression. M, DL2000 marker kit (marker size as same as the Fig.1); 1, liver; 2, muscle; 3, ovary; 4, small intestine; 5, heart; 6, fat; 7, lung; 8, kidney; 9, spleen

Tissue expression: The RT-PCR profiling of tissue expression of the swine PFKFB1 gene was carried out using samples from one adult Wujin x Large white cross pig. The swine PFKFB1 gene is predominantly expressed in muscle, heart, lung, liver, small intestine and kidney and weakly expressed in fat, ovary and spleen (Fig. 6).

PFKFB1, this gene encodes a member of the family of bifunctional 6-phosphofructo-2-kinase:fructose-2,6-bi-phosphatase enzymes. The enzyme forms a homodimer that catalyzes both the synthesis and degradation of fructose-2,6-biphosphate using independent catalytic domains. Fructose-2,6-biphosphate is an activator of the glycolysis pathway and an inhibitor of the gluconeogenesis pathway. Consequently, regulating fructose-2,6-biphosphate levels through the activity of this enzyme is thought to regulate glucose homeostasis (Algaier and Uyeda, 1988; Lee *et al*., 2003; Veech, 2003). To this date, the PFKFB1 gene was indentified and characterized in horse, dog, human, olive baboon, red-bellied titi and other animals; the swine PFKFB1 has not been reported yet.

CONCLUSION

The present results show that the PFKFB1 gene is differentially expressed in the longissimus dorsi muscle being a more abundant in Large white than in Wujin pigs. Wujin is a fat type pig breed comprising much more body fat than lean meat/muscle. On the other hand, Large white is a typical lean type pig breed, presenting the opposite phenotype to that described for the Wujin breed. The two pig breeds used in this study, Large white and Wujin, differ in lean meat percentage.

It is therefore, interesting that the expression of the swine PFKFB1 gene in the longissimus dorsi muscle shows the trend of a higher expression in Large white as compared with Wujin.

A major question is the extent to which such predominant expression could be developmentally or metabolically significant in terms of acquiring of any phenotypic change in favor of a higher lean-type rate. Clearly, this merits needs further study.

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