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Identification of a Differentially Expressed Gene *PPP1CB* between Porcine *Longissimus dorsi* of Meishan and Large White×Meishan Hybrids

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Abstract To study the molecular basis of heterosis, suppression subtractive hybridization was used to investigate the differences in gene expression between porcine *Longissimus dorsi* of F1 hybrids Large White×Meishan and their female parents Meishan. From two specific subtractive cDNA libraries, the clones selected by reverse Northern high-density blot screening were chosen to clone full-length cDNA by rapid amplification of cDNA ends. An expression-upregulated gene for Meishan skeletal muscle, designated protein phosphatase 1, catalytic subunit, beta isoform (*PPP1CB*), was identified. Porcine *PPP1CB* contains an open reading frame encoding 327 amino acid residues with 13 and 1763 nucleotides in the 5′ and 3′ untranslated regions, respectively. A DNA fragment of 721 nucleotides was amplified and a mutation that creates/disrupts a restriction site for endonuclease *Rsa*I was found. The derived amino acid sequence of *PPP1CB* has high homology with the *PPP1CB* of three species, *Mus musculus* (99%), human (99%) and mouse (100%). The tissue expression analysis indicated that the swine *PPP1CB* gene is generally expressed in most tissues. The possible role of *PPP1CB* and its relation to porcine heterosis are discussed.

Key words differential gene expression; pig; heterosis; *PPP1CB*; suppression subtractive hybridization

The use of heterosis has achieved great success in agriculture and is considered essential to meet the world's food needs [1,2]. To reveal the mechanism of heterosis, many hypotheses have been advanced, such as the dominance, overdominance and epistasis hypotheses [3], intergenomic complementation [4], the nucleo-cytoplasmic interaction and genetic equilibrium hypotheses [5], and the concerted effect of heterozymes [6]. But none of these can perfectly explain heterosis.

The phenomenon of heterosis is in fact the external exhibition of gene expression and regulation in the heterozygote [7]. It is necessary to go one step further to understand the molecular mechanisms of heterosis in terms of hybrids' differential gene expression relative to their parents. Recently, by differential display of mRNA and suppression subtractive hybridization (SSH), we have detected significant differences in mRNA quantity and their expressed patterns between porcine F1 hybrids and their parents [8,9]. Thus, cloning and characterization of genes that are differentially expressed between hybrids and their parents should provide further insight into the molecular mechanisms responsible for heterosis. Here we report the identification of a differentially expressed gene *PPP1CB* in the *Longissimus dorsi* between F1 hybrids of Large White×Meishan and their female parents Meishan by SSH.

The reversible phosphorylation of proteins, catalysed by protein kinases and protein phosphatases, is a major mechanism for the regulation of almost all cellular functions, from metabolism to signal transduction, cell division and memory [10]. Phosphatases are classified into two major functional groups, protein tyrosine phosphatases and protein Ser/Thr phosphatases, although functional overlap of various extents is sometimes encountered [11, 12].

The physiochemical properties of Ser/Thr phosphatases

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were used to classify these enzymes into four major classes: PPP1, PPP2A, the Ca^{2+}-calmodulin regulated phosphatase (PPP2B) and the Mg^{2+}-dependent phosphatase (PPP2C). The catalytic subunits of PPP1 (PPP1C), PPP2A (PPP2AC) and PPP2B belong to the same gene family sharing approximately 40% sequence identity with no structural similarity to PPP2C or protein tyrosine phosphatases [13].

There are four PPP1 isoforms: PPP1α, PPP1β, and two forms of PPP1γ, termed PPP1γ1 and PPP1γ2 [14]. PPP1 regulates many diverse cellular processes [15]. It is the major phosphatase that regulates glycogen metabolism in response to insulin and adrenalin [16]. PPP1 also controls the activity of the sarcoplasmic reticulum Ca^{2+}-ATPase [17], smooth muscle contraction and protein synthesis [18]. However, relatively little is known about the porcine PPP1C.

In the present study, we describe the cloning and expression profile of porcine PPP1C. Additionally, part of the genomic sequence and polymorphisms of porcine PPP1C were cloned and characterized.

Materials and Methods

Animals

All animals used in the study were derived from the cross-experiments conducted in Huazhong Agriculture University Jingpin Pig Station (Wuhan, China).

SMART cDNA synthesis, SSH and reverse Northern screening

Total RNAs were isolated with Trizol Reagent (Gibco, Grand Island, USA) from the *Longissimus dorsi* of six F1 hybrids of Large White×Meishan (three male and three female) of 4 months old, and six pigs (three male and three female) at the same age of Meishan, the female parent of the cross-experiment, and were mixed to form RNA pools. Poly(A)$^+$ RNA was purified by biotinylated oligo (dT) probe and streptavidin-bound magnetic particles (Promega, Madison, USA). SMART cDNA was synthesized using the SMART PCR cDNA Synthesis Kit (Clontech, Palo Alto, USA) for rapid amplification of cDNA end (RACE)-polymerase chain reaction (PCR). SSH was carried out as described in the PCR-Select cDNA Subtraction Kit (Clontech). In the forward subtraction, the cDNA from Meishan was used as the tester, and the cDNA from Large White×Meishan hybrid as the driver; in the reverse subtraction, the cDNA from Large White×Meishan hybrid was used as the tester, and the cDNA from Meishan as the driver. The differentially expressed PCR products were inserted into pGEM-T vector (Promega) and transformed into *Escherichia coli* DH5α, then cultured on LB plates containing 50 μg/ml of ampicillin, 0.4 mM of X-gal and 0.4 mM of isopropyl β-*D*-thiogalactopyranoside. White colonies were picked up and 1 μl of each bacterial LB culture was amplified in a 25 μl PCR system using nested PCR primers 1 and 2R (Clontech). PCR products were then denatured and alkaline blotted onto two Hybond-N$^+$ positively charged nylon membranes (Roche, Mannheim, Germany) in parallel [19]. The two nylon membranes were hybridized with forward-subtracted and reverse-unsubtracted probes. Reverse Northern high-density blot screening was carried out according to the procedure of the DIG High Prime Labeling and Detection Starter Kit I (Roche). The positive clones were sequenced using vector-specific primers.

Reverse transcription (RT)-PCR

Total RNA derived from the *Longissimus dorsi* of additional three F1 hybrids of Large White×Meishan and their female parents Meishan was used as the starting material, and cDNA was synthesized as the PCR template using Moloney murine leukemia virus reverse transcriptase and the oligo(dT) primer (Promega). A specific primer pair G3PDH-F/G3PDH-R (**Table 1**) that amplified the 476 bp housekeeping gene *G3PDH* was used as the internal control. The differentially expressed clone, *MS140*, was detected by the specific primers 140F and 140R (**Table 1**).

For spatial expression analysis of *MS140*, total RNA was also isolated from various tissues (heart, liver, spleen, lung, kidney, adipose tissue, *Longissimus dorsi*, embryo,

Table 1 Primers used in this study for sequencing and expression analysis

Name	Sequence (5′→3′)
SMART 5′	ACGCAGAGTACGCGGG
SMART 3′	CAGAGTAC$(T)_{16}$
140-1F	AGCCATTTGACACCCTTTAT
140-1R	TCTTCCCACATTTCACTCTA
140-2F	AAATATGCAAACTGTTCGAC
140-2R	CTCAAAACAAAAGAGAACAA
140-WF	CTTTCTGGTAACACCCTCA
140-WR	GTGACATAAGCCTTACAATC
G3PDH-F	AATTTCGCACAACTTCAG
G3PDH-R	TGGAAAGATAGCAGAGGC

testis, uterus, ovary) and cDNAs were synthesized.

RACE-PCR

To obtain the 5′ full-length cDNA, an oligonucleotide primer 140-1R (**Table 1**) complementary to the 3′ end of the cDNA was used in combination with the SMART 5′ primer (**Table 1**), designed against SMART II oligonucleotide from the SMART PCR cDNA Synthesis Kit to amplify the 5′ end of the gene, using SMART amplified cDNA from pigs' *Longissimus dorsi* as the template. PCR was carried out in a GeneAmp PCR System 9600 (Perkin Elmer, Ramsey, USA) with the following cycling parameters: 95 ºC initial denaturation for 4 min; 35 cycles of 95 ºC denaturation for 40 s, 56 ºC annealing for 40 s, and 72 ºC extension for 1.5 min; followed by a 10 min extension at 72 ºC.

To obtain the 3′ full-length cDNA, primer 140-1F (**Table 1**) was used in combination with the SMART 3′ primer (**Table 1**), designed against CDS III 3′ PCR Primer from the SMART PCR cDNA Synthesis Kit to amplify the 3′ end of the cDNA. PCR was carried out with the cycling parameters: 95 ºC initial denaturation for 4 min; 35 cycles of 95 ºC denaturation for 50 s, 54 ºC annealing for 50 s, and 72 ºC extension for 2 min; followed by a 10 min extension at 72 ºC.

Database and sequence analysis

The full-length nucleotide sequence of the porcine *PPP1CB* was compared with GenBank at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/Genbank/index.html) using BLASTN and BLASTX searches of the "nr" database. The deduced amino acid sequence was analyzed using the ExPASy Molecular Biology Server (http://cn.expasy.org/). Multiple sequence alignments and construction of the unrooted phylogenetic tree were carried out using the CLUSTALW 1.83 program (http://www.ebi.ac.uk/clustalw/index.html).

Genomic PCR and identification of gene polymorphisms

One primer pair (140-2F and 140-2R) was designed (**Table 1**) to amplify a fragment of the *PPP1CB* gene from the porcine genome. The optimal cycling parameters were 95 ºC initial denaturation for 4 min; 35 cycles of 95 ºC denaturation for 40 s, 55 ºC annealing for 40 s, and 72 ºC extension for 45 s; followed by a 10 min extension at 72 ºC. The products were cloned into the pGEM-T cloning vector and sequenced using vector-specific primers. Polymorphisms were detected based on sequence comparison.

Results

Identification of *MS140* as an upregulated gene in *Longissimus dorsi* from Meishan

In order to isolate differential genes between F1 hybrids of Large White×Meishan and their female parents Meishan, forward and reverse subtractive cDNA libraries were constructed. By reverse Northern high-density blot screening of more than 600 clones randomly picked up from subtractive libraries, one clone, designated *MS140*, demonstrated high-level expression in *Longissimus dorsi* for Meishan [**Fig. 1(A)**]. RT-PCR analysis was used to confirm differential expression of *MS140* [**Fig. 1(B)**].

Cloning of the full-length cDNA of porcine skeletal muscle

The differentially expressed clone (*MS140*) was found to have 97% homology to the mRNA sequence of *Bos taurus* gene for Ser/Thr protein phosphatase PPP1β catalytic subunit. Further sequence analysis indicated that the

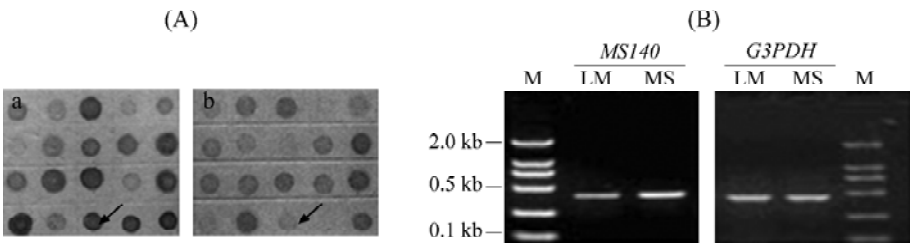

Fig. 1 Identification of MS140 as the upregulated gene in *Longissimus dorsi* muscle from Meishan pigs by reverse Northern high-density blot screening (A) and reverse transcription-polymerase chain reaction (RT-PCR) (B)

(A) Reverse Northern high-density blot screening. Two identical membranes were probed with secondary PCR products enriched from forward subtractive Meishan cDNA. a, reverse unsubtractive Large White×Meishan cDNA; b, MS140 is indicated with an arrow. (B) RT-PCR analysis of MS140. M, marker; LM, F1 hybrids Large White×Meishan; MS, female parents Meishan.

cDNA fragment of MS140 (GenBank accession No. DQ396472) contained part of the CDS and part of the 3′ untranslated region (UTR) of the cDNA (72 bp of predicted translated sequence+525 bp UTR=597 bp in total). One PCR fragment of approximately 1.5 kb was amplified by 5′-RACE [**Fig. 2(A)**]. There were several fragments amplified by 3′-RACE and the brightest one of approximately 1.7 kb was reamplified [**Fig. 2(B)**]. These products were then cloned to T vectors and sequenced. A 2759 bp complete cDNA sequence of *PPP1CB* was identified (GenBank accession No. DQ396471) and shared a 100% similarity with the CDS of swine PPP1CB (NM214184). Porcine *PPP1CB* cDNA contains an open reading frame of 984 nucleotides, encoding a protein of 327 amino acids (**Fig. 3**). We inferred the ATG codon at nucleotide 14–16 to be the true start site of translation, because it begins the longest reading frame, which is homological to that of other species. In addition, the putative methionine initiation codon occurs in a favorable sequence context for the initiation of translation with a purine in the –3 position and a G in the +4 position [20]. A polyadenylation signal, AATAA, was found at nucleotide 2272–2276 located upstream of the poly(A) tract. Another polyadenylation signal, AGTAAA, was found just before the poly(A) tract. Five AU motifs (ATTTA) associated with the instability of mRNA were found in the 3′ UTR.

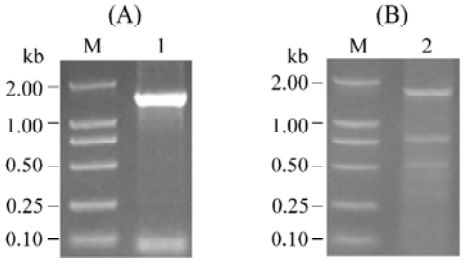

Fig. 2 Polymerase chain reaction fragments amplified by 5′-rapid amplification of cDNA end (RACE) and 3′-RACE for gene *PPP1CB*

M, DL2000 marker; 1, 5′-RACE product; 2, 3′-RACE product.

Analysis of the *PPP1CB* protein sequence

The conceptual translation product of the porcine *PPP1CB* transcript is a peptide of 327 amino acids. Comparison with three reported molecules of other species shows an extraordinary high level of similarity (99%–100% identity overall). The amino acid sequence of *PPP1CB* is highly conserved among species. The differences between the protein encoded by this gene in pig and three other spieces are shown in **Table 2**.

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GAGTGCCGACAAGATGGCGGACGGGGAGCTGAACGTGGACAGTCTCATCACCCGACTGCTGGAGGTACGA   70
        M  A  D  G  E  L  N  V  D  S  L  I  T  R  L  L  E  V  R           19
GGATGTCGTCCAGGAAAGATTGTGCAGATGACTGAAGCAGAGGTTCGGGGTTTATGTATCAAGTCTCGGGAG  142
 G  C  R  P  G  K  I  V  Q  M  T  E  A  E  V  R  G  L  C  I  K  S  R  E    43
ATCTTTCTCAGCCAGCCTATTCTTTTGGAATTGGAAGCACCACTGAAGATTTGTGGAGATATTCATGGACAG  214
 I  F  L  S  Q  P  I  I  I  F  I  E  A  P  I  K  I  C  G  D  I  H  G  Q    67
TATACAGATTTACTGCGATTATTTGAATATGGAGGTTTCCCACCAGAGGCCAACTATCTTTTCTTAGGAGAT  286
 Y  T  D  L  L  R  L  F  E  Y  G  G  F  P  P  E  A  N  Y  L  F  L  G  D    91
TATGTAGACAGAGGAAAGCAGTCTTTGGAAACCATTTGTTTGCTGCTGGCTTATAAAATCAAATATCCAGAG  358
 Y  V  D  R  G  K  Q  S  L  E  T  I  C  L  L  L  A  Y  K  I  K  Y  P  E   115
AACTTCTTTCTCTTAAGAGGAAACCATGAGTGTGCTAGCATCAATCGCATTTATGGATTCTATGATGAATGC  430
 N  F  F  L  L  R  G  N  H  E  C  A  S  I  N  R  I  Y  G  F  Y  D  E  C   139
AAACGAAGGTTTAATATTAAATTGTGGAAGACCTTCACTGATTGTTTCAACTGCTTGCCTATAGCTGCCATT  502
 K  R  R  F  N  I  K  L  W  K  T  F  T  D  C  F  N  C  L  P  I  A  A  I   163
GTGGATGAGAAGATCTTCTGTTGTCACGGAGGACTGTCACCAGACCTGCAGTCTATGGAGCAGATTCGGAGA  574
 V  D  E  K  I  F  C  C  H  G  G  L  S  P  D  L  Q  S  M  E  Q  I  R  R   187
ATCATGAGACCCACTGATGTCCCTGATACAGGTTTGCTCTGTGATTTGCTGTGGTCTGACCCAGATAAGGAT  646
 I  M  R  P  T  D  V  P  D  T  G  L  L  C  D  L  L  W  S  D  P  D  K  D   211
GTGCAAGGTTGGGGAGAAAATGACCGTGGTGTTTCCTTCACTTTTGGAGCTGATGTAGTCAGTAAATTTCTG  716
 V  Q  G  W  G  E  N  D  R  G  V  S  F  T  F  G  A  D  V  V  S  K  F  L   235
AATCGTCATGATTTAGACTTAATTTGTCGAGCTCATCAGGTGGTGGAAGATGGATATGAATTCTTTGCTAAA  790
 N  R  H  D  L  D  L  I  C  R  A  H  Q  V  V  E  D  G  Y  E  F  F  A  K   259
CGACAATTGGTAACCCTATTTTCAGCCCCAAATTACTGTGGCGAGTTTGATAATGCTGGTGGAATGATGAGT  862
 R  Q  L  V  T  L  F  S  A  P  N  Y  C  G  E  F  D  N  A  G  G  M  M  S   283
GTGGATGAAACTTTGATGTGTTCATTTCAGATATTGAAACCATCTGAAAAGAAAGCTAAGTACCAATATGGT  934
 V  D  E  T  L  M  C  S  F  Q  I  L  K  P  S  E  K  K  A  K  Y  Q  Y  G   307
GGACTGAATTCAGGACGTCCTGTCACTCCACCTCGAACAGCTAATCCTCCGAAGAAAAGGTGAAGAAAGGAA 1006
 G  L  N  S  G  R  P  V  T  P  P  R  T  A  N  P  P  K  K  R  *            327
CTCTGTAAAGAAACCATCAGATTTGTTAAGGACATACTTCATGATATATAAGTGTGCACTGTAAAACCATCC 1078
AGCCATTTGACACCCTTTATGATGTCACACCTTTAACTTAAGGAGACGGGTAAAGGATCTTAAATTTTTTTC 1150
TAATAGAAAGATGTGCTACACTGTATTGTAATAAGTATACTCTGTTAAAATATTCAACAAAGTTAAATCTAA 1222
ATTCAGACTTACCCATTAAAGTTACATCTTCACGTATCAGTTTTTAAAGTTGAAAAGCATCCCAGTTAAACT 1294
AGATGTGATAGTTAAACCAGATGAAAGCATGATGATCCATCTGTGTAATGTGGTTTTAGTGTTGCTTGGTTG 1366
TTTAATTATTTTGGGCTTGTTTTGTTTTGTTTGTTTTTGCTAGAATAATGGCAGATTCTTTAAATTTTTTTC 1438
CCAAACATCCTTAGAGTGAAATGTGGGAAGAATTTTAAAGACGTTGATTGGCTATTATTTTCCCTTGCTTAT 1510
TTACTTATGTACCTGTTTGATCTTACTGAGAAAACTTAATGCCTCATAACGGTAAAAAGGAATTTTAGAGAT 1582
ATTTTTTAAAAATATGCAAACTGTTCGACCGAGTG***ATTTTA***ATCAGTTTGGGCAAATGTTACAGCTGATAATG 1654
AATATTTTGCTTTATACAGAAATTGCCACTGATTTGGATTTGTGCACTCTAATTTTAACTTATTGATGCTCT 1726
ATTGTGCAGTAGCATTTC***ATTTA***ACTTTAAGATAAGGCTC***ATTTA***GTATACCCAACTAGTTGGTAATGTGAT 1798
TATGTGGTACCTTGGCTTTAGGTTTTAATTCGCACGGAACACCTTTTTGGCATGCTTAACTTTCTGGTAACA 1870
CCCTCACCTGCATCGGTTTCGGTTTTTTTTGTTTGTTTGTTTTGTTTTGTTTGGGGGGGTTTTTTGTTTGTT 1942
TGTGTGCGTGTGTGTTGTTTTTTTGTTTTTAGATCCACAAAACATGAGAATCCTTTTTTGACGAGCCTTG 2014
GAAAGCTGACACTATCTCTTTTTTCCTCCCTCTGTACGAAGGATGT***ATTTA***AATGAATGCTGGTCAGTGGGA 2086
CGTTTGTCAACTCTGGGTATTGGGTGCTTTTAACTGTCTAATATTGCCATGTGAATGTTGTATACGATTGTA 2158
AGGCTTATGTCACTAAAGATTTTTATTCTGATTTTTCATGATCAAAGTTCATATGATATTGTATAGACATGC 2230
TTTGTAGTGAACTATAGTAGCAATAATTTCTGTACATGATCAAGGGTTTATTGCAGCATTTCTTGTTCTCTT 2302
TTGTTTTGAGGGTTAGTATTAACAAATGACAGGAATAGAAAAATCAGCATAAAGATTTTAGAAaGGAGAACT 2374
TAAAGGACACAGATTTGTGATTCcTTTGGATGCAACACTTTTGGATGTGATTCTAAAAGCTTTTATTGAGCA 2446
TTGTCAAATTTCTATGCTTCACAGGCACAGACATCAT***ATTTA***TAATACCCTTCTATAAGTGCTACCATAGAT 2518
GTGAAATTCTTGACCTTAATATTGTCTTTGAAAATGTTAAATTGAGAATTCTGTTAACTTACATTTTAAGAA 2590
TTGGCATATTGTATTGCTGCAAGAGATTTCATTTTCAGCACAGTGCAAAGTTCTTTTAAATGCATATGTCTT 2662
TTTTTTCTAATTCCATTTTGTTTTAAAGCACATTTTAAATGTAGTTTTCTC***ATTTA***GTAAAAGTTGTCTAAT 2734
CGAAAAAAAAAAAAAAAAAAAAAAA                                               2759
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Fig. 3 Nucleotide and predicted amino acid sequences of porcine *PPP1CB*

The start codon (ATG) is boxed and the stop codon (TAG) is indicated with an asterisk. The polyadenylation signal (AATAA, AGTAAA) is underlined. The AU motifs (ATTTA) are indicated in bold and italics. The SNP site is shaded.

Table 2 Differences in amino acids (a.a.) between the *PPP1CB* gene of pig and three other species

Species	Serial number of a.a.					
	1	·····51	·····69	·····264	·····320	·····327
Sus scrofa	M	·····L	·····T	·····T	·····A	·····R
Mus musculus	M	·····L	·····T	·····T	·····A	·····R
Homo sapiens	M	·····P	·····T	·····T	·····A	·····R
Canfa	M	·····L	·····A	·····T	·····D	·····R

Analysis of phylogenetic tree

A phylogenetic tree was constructed using the porcine *PPP1CB* sequence from our study and other recently available sequences in the database, using DNASTAR software, as shown in **Fig. 4**. The phylogenetic tree analysis revealed that the swine *PPP1CB* has a closer genetic relationship with the *PPP1CB* of gallus, rabbit, rat, chick, than with those of human, mouse, dog, frog and danio.

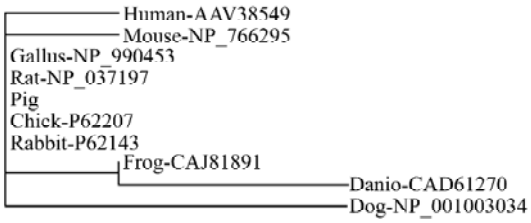

Fig. 4 Phylogenetic tree for ten kinds of *PPP1CB*

The sequences used for analysis are derived from GenBank, and their accession No. are shown on the right-hand side.

Tissue expression profile

The RT-PCR analysis of the tissue expression profile was carried out using the tissue cDNAs of pigs as the templates, and the result revealed that the swine *PPP1CB* gene was not only expressed in *Longissimus dorsi* muscle, but also expressed in the heart, liver, spleen, lung, kidney, adipose tissue, testis, uterus and embryo (**Fig. 5**).

Polymorphisms of the porcine *PPP1CB*

To search for different *PPP1CB* alleles within the pig populations, part of the 3′ UTR of *PPP1CB* was investigated in three different individuals representing three pig breeds (Large White, Landrace and Chinese Meishan). A DNA fragment of 721 nucleotides was amplified and sequenced (GenBank accession No. DQ398872). Two

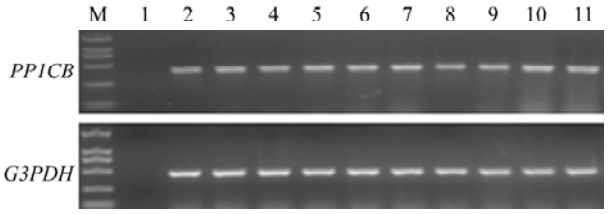

Fig. 5 Tissue expression profile analysis of the porcine *PPP1CB* gene

M, DL2000 marker; 1, water; 2, adipose tissue; 3, *Longissimus dorsi*; 4, embryo; 5, testis; 6, uterus; 7, kidney; 8, lung; 9, heart; 10, spleen; 11, liver.

potential polymorphic sites were identified and a mutation created/disrupted a restriction site for endonuclease *Rsa*I (**Fig. 6**). We analysed this polymorphism in purebreds and crossbreds (Large White, Meishan, Landrace and Large White×Meishan) by means of the PCR-restriction fragment length polymorphism technique (**Fig. 7**) using primer pairs 140-WF and 140-WR (**Table 1**). Allele AA was not found.

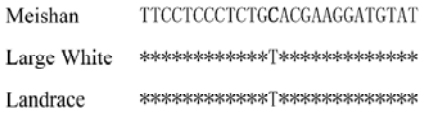

Fig. 6 A mutation in the 3′ untranslated region

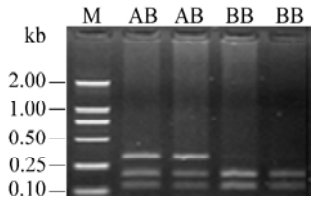

Fig. 7 Restriction pattern of the amplification with primers 140-WF and 140-WR digested with *Rsa*I

M, DL-2000 DNA molecular weight marker; AB, genotype AB; BB, genotype BB.

Allele BB was highly frequent in Meishan and Landrace. Allele AB was highly frequent in F1 hybrids Large White×Meishan (**Table 3**).

Table 3 Polymorphism in purebred and crossbred pig populations by digesting the amplification of primers 140-WF and 140-WR with endonuclease *Rsa*I

Population	*n*	Genotype		
		AA	AB	BB
Large White	30	0	17	13
Landrace	28	0	8	20
Meishan	24	0	2	22
Large White×Meishan	23	0	20	3

Discussion

SSH is a recently developed method for identifying differentially expressed genes between two different mRNA populations. The efficiency and reproducibility of SSH has been proven in different studies for differentially expressed genes. SSH combined with the high throughput reverse Northern screening method permits the efficient and rapid cloning of dozens to hundreds of differentially expressed genes in one experiment [21]. It was reported that the rate range of the positive cDNA clones obtained by SSH was from 10% to 90% [22], which is higher than differential display PCR [23] and representational difference analysis [24] methods.

The level of protein phosphorylation is controlled by the opposing and coordinated activities of protein kinases and phosphatases that catalyse protein phosphorylation and dephosphorylation, respectively. PPP1 is a major eukaryotic protein Ser/Thr phosphatase that regulates diverse cellular processes such as cell-cycle progression, protein synthesis, muscle contraction, carbohydrate metabolism, transcription and neuronal signaling [25]. Therefore the activity of PPP1C including *PPP1CB* is very important for protein phosphorylation and the regulation of many physiological processes. Thus, the downregulated expression observed in the F1 hybrids suggests that the phosphorylation process, mediated through *PPP1CB*, might be a candidate molecular pathway for influencing porcine heterosis. In addition, in Meishan pigs, there is a high frequency of the B allele, and 22 of 24 animals are BB genotypes (**Table 3**). Meishan is also the breed showing the higher level of expression of *PPP1CB*, so it is also possible that there is a link between the presence of the B allele and the upregulation of gene expression in Meishan pigs. Of course, it is also possible that *PPP1CB* downregulated expression is linked to a quantitative trait locus and that the amount of *PPP1CB* mRNA has no direct effect on porcine heterosis. All of these points need further verification.

It would be premature to speculate how changes in *PPP1CB* in hybrids might affect heterosis, but alterations in the reversible phosphorylation system might be important for other modifications in patterns of gene expression in hybrids that affect heterosis in their turn. To further understand the function of the gene, more research based on these primary results is needed.

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