



# Molecular characterization, tissue expression profile, and SNP analysis of porcine *SLC13A5*

L.Y. Wang, J. Jiang and H.M. Ma

College of Animal Science and Technology of Hunan Agricultural University,  
Changsha, China

Corresponding authors: J. Jiang / H.M. Ma  
E-mail: jiangjun1121@126.com / mahaiming2000@163.com

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**ABSTRACT.** Solute carrier family 13 (sodium-dependent citrate transporter member 5, *SLC13A5*) gene has been recently found to play an important role in intramuscular fat content in pigs. In this study, the full-length cDNA of porcine *SLC13A5* was obtained from the longissimus dorsi muscle of Shaziling pigs, using the rapid amplification of cDNA ends technique. Full-length porcine *SLC13A5* cDNA was 2118 bp, with a 1665-bp open reading frame encoding 554 amino acids. The porcine *SLC13A5* protein was analyzed using bioinformatic methodology, and found to include 18 potential phosphorylation sites (including six serine, nine threonine, and three tyrosine) and eight putative transmembrane domains. One single nucleotide polymorphism (SNP) site, A251G, was identified by polymerase chain reaction (PCR)-restriction fragment

length polymorphism and the associations of this SNP with age at 100 kg and corrected back fat thickness were found to be not significant. Expression of *SLC13A5* was evaluated in ten tissues from 25-day-old full-sib Yorkshire and Shaziling piglets (both N = 3), using quantitative PCR analysis. Expression levels of *SLC13A5* differed significantly between the breeds in cecum, liver and crureus muscle. In each breed, gene expression levels were significantly different in longissimus dorsi muscle, compared to the nine other tissues. This study has laid the foundation for further investigations of the molecular mechanisms of *SLC13A5* in pigs.

**Key words:** Pig; *SLC13A5*; Cloning; Single nucleotide polymorphism; Expression profiles

## INTRODUCTION

It has been previously demonstrated that the solute carrier family 13 member 5 (*SLC13A5*) gene family consists of five related members including *SLC13A1*, *SLC13A2*, *SLC13A3*, *SLC13A4*, and *SLC13A5*. It have been described in a variety of animals, plants, yeast, and bacteria (Shulman and Helfand, 2011; Birkenfeld et al., 2011; Martinez-Beamonte et al., 2011). *SLC13A5* is a sodium- coupled transporter that mediates cellular uptake of citrate, which plays important role in the synthesis of fatty acids (Li et al., 2015). Mammalian solute carrier family 13 mediates transport of Na<sup>+</sup>-coupled anion at the plasma membrane of epithelial (kidney, small intestine, placenta, and liver) (Pajor, 2006; Bergeron et al., 2013). CpG sites of *SLC13A5* in glioblastomas exhibited that expression level had a negative correlation with promoter methylation (Etcheverry et al., 2010).

In recent years, more and more researches have been focused on the meat quality. It is a very complicated trait because this trait is made up with many components including the intramuscular fat content (IMF) (Chen et al., 2013; Ros- Freixedes et al., 2014; Sato et al., 2014; Ren et al., 2014). IMF is one of the most important factors affecting meat juiciness, tenderness, and flavor (Schwab et al., 2006; Wood et al., 2004, 2008; Madeira et al., 2013). Estimated heritability of the IMF was 0.78 (Biermann et al., 2015). Schworer and Lorenz (1995) reported that the selection of high IMF in pigs is necessary to improve pork quality. In pigs the *H-FABP* gene contributed to IMF and it has been applied to breeding programs of pigs (Ding et al., 2011).

The porcine *SLC13A5* gene is located on *Sus scrofa* chromosome 12 (SSC12) ([http://www.ncbi.nlm.nih.gov/projects/mapview/map\\_search.cgi?taxid=9823&build=2.1](http://www.ncbi.nlm.nih.gov/projects/mapview/map_search.cgi?taxid=9823&build=2.1)). There are three quantitative trait loci for IMF on SSC12 (Xiong et al., 2012; Ma et al., 2009, 2013). Moreover, Luo et al. (2012) used a genome-wide association study (GWAS) method to determine that porcine *SLC13A5* was significantly related to IMF.

In the current study, in order to investigate whether *SLC13A5* expression was associated with IMF in pigs, the cDNA sequence of *SLC13A5* was cloned by rapid amplification of cDNA ends (RACE). The characteristics of the *SLC13A5* protein were evaluated using bioinformatic analysis, and expression levels in ten tissues from two pig breeds were determined by quantitative real-time PCR. The aim of this research was to reveal the influence of *SLC13A5* on fat deposition and provide further insights into the mechanism of porcine *SLC13A5* in meat quality trait.

## MATERIAL AND METHODS

### Animals and samples

Animals were reared in Taoyuan, Ningxiang, Xiangtan, and the Changsha county of Hunan Province, China. Ear tissue samples were collected from adult Ningxiang (N = 65), Yorkshire (N = 330), Taoyuan black (N = 112), Shaziling (N = 57), and Daweizi (N = 80) pigs and genomic DNA extractions was performed. Samples of longissimus dorsi, crureus, lung, liver, pancreas, intestine, cecum, heart, spleen, and kidney were collected from 25-day-old full-sib Shaziling and Yorkshire pigs (both N = 3), immediately frozen in liquid nitrogen, and then stored at -80°C until further use.

### RNA extraction, reverse transcription, cloning and sequencing

Total RNA was extracted from longissimus dorsi muscle of Shaziling pigs using an RNA extraction kit (Takara, Shiga, Japan) in accordance with the manufacturer instructions. The quality and concentration of RNA in each sample were evaluated by Nanodrop 2000 spectrophotometer. First-strand cDNA was produced using the Superscript II RT reverse transcription system kit (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer protocol. Amplification by PCR was conducted under the following conditions: 94°C for 5 min; 32 cycles of 94°C for 20 s, 55°C for 25 s, and 72°C for 30 s; and 72°C for 5 min. Following gel purification, RT-PCR products were cloned into a pMD19-T vector (Takara) and sequenced.

### Cloning of porcine *SLC13A5*

To obtain full-length porcine *SLC13A5* cDNA, two primer pairs were designed to amplify 5'- and 3'-ends of *SLC13A5* gene, by RACE, and obtained a coding sequence (CDS) of porcine *SLC13A5*. Amplifications by 5'- and 3'-RACE PCR were performed using a SMART™ RACE cDNA amplification kit (Clontech Company, Madison, WI, USA) and the manufacturer protocol. Gene-specific (GSPs), universal (UPM) and abridged universal amplification (AUAP) primers are presented in Table 1. Amplified products were separated by electrophoresis on a 1.5% agarose (w/v) gel. Following gel purification, RT-PCR products were cloned into a pMD18-T vector (Takara) and sequenced, using the DNASTar 7.1 software (<http://www.gpox.com>) to obtain full-length cDNA sequence. Sequence homologies were performed by comparisons with published sequences, using the NCBI-BLAST database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

### Bioinformatic analysis

Analysis of cDNA and amino acid sequence deduction were performed with the Expert protein analysis system (<http://www.expasy.org/>). Amino acid sequences were aligned using CLUSTALW version 2.0 (<http://www.simgene.com/ClustalW>), and protein domain features was predicted using the simple modular architecture tool (<http://smart.embl-heidelberg.de/>). Phosphorylation sites were analyzed by NetPhos2.0 (<http://www.cbs.dtu.dk/services/NetPhos/>). A phylogenetic tree was constructed with *SLC13A5* full-length cDNA sequences of different species using the neighbor-joining method and the MEGA version 5.0 software (Tamura et al., 2011).

### Real-time quantitative PCR (qPCR) analysis

To evaluate the expression of porcine *SLC13A5* in different tissues, RNA was isolated from ten different tissues using an RNA extraction kit and following the manufacturer instructions (Takara). Total cDNA was generated using a QuantiTect transcription kit (Takara). Primers of SYBR green were designed using the Primer premier 5.0 software (Table 1). Real-time PCR was carried out using a BIO-RAD iCycler Thermal Cycler w/iQ5 (Hercules, CA, USA). Results were calculated using the relative quantification method and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as an internal control gene. Expression levels of *SLC13A5* were calculated using the  $2^{-\Delta\Delta Ct}$  comparative CT method ( $\Delta\Delta Ct = \Delta Ct_{\text{target gene}} - \Delta Ct_{\text{housekeeping gene}}$ ). Amplification reactions were carried out in a final volume of 20  $\mu\text{L}$ , comprising 10  $\mu\text{L}$  2X qPCR Mix (Takara); 1  $\mu\text{L}$  of each primer (10  $\mu\text{M}$ ); 1  $\mu\text{L}$  template cDNA; and 8  $\mu\text{L}$  ddH<sub>2</sub>O, according to the following program: 95°C for 2 min, followed by 35 cycles of 95°C for 10 s, 60°C for 40 s and 72°C for 30 s.

### Single nucleotide polymorphism (SNP) identification and allele frequency analysis of porcine *SLC13A5*

Pig genomic DNA was extracted by the phenol/chloroform method from ear tissue samples, and used as template DNA for amplification using five primer pairs (Table 1) to detect SNPs. Genomic DNA was amplified by PCR, and then PCR products were directly sequenced to detect mutations. A polymorphism site was identified using the Seqman program of DNASTar 7.1 software (<http://www.gpax.com>). Genotyping was based on genomic DNA of all breeds using the PCR-restriction fragment length polymorphism technique with *Bsu36I* restriction enzyme (Fermentas, Beijing, China). Amplified PCR products were digested at 37°C for 10 h in a mixture (20  $\mu\text{L}$ ) containing 9.8  $\mu\text{L}$  PCR products; 8  $\mu\text{L}$  nuclease-free water; 2  $\mu\text{L}$  10X Buffer Tango (Promega, Madison, WI, USA); and 0.2  $\mu\text{L}$  *Bsu36I* (10 U).

## RESULTS

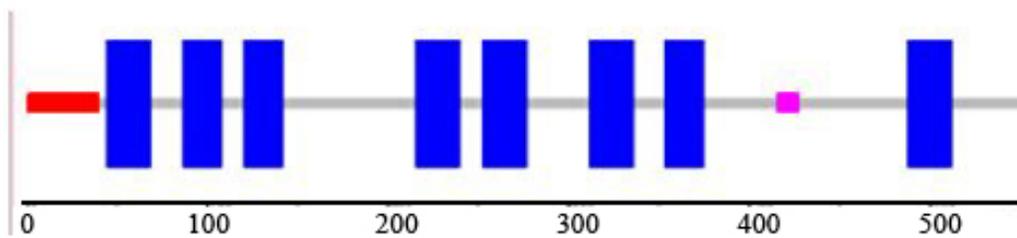
### cDNA cloning and sequence analysis of porcine *SLC13A5*

The complete cDNA sequence of *SLC13A5* from Shaziling pigs was obtained by RACE and was 2118 bp long. Porcine *SLC13A5* sequence was deposited in GenBank (accession No. KF728381). The total cDNA length and deduced amino acid sequences are displayed in Figure 1. The nucleotide sequence of *SLC13A5* contained an open reading frame that was 1665 bp in length and encoded 554 amino acids, and the 5'- and 3'-untranslated regions were 557 and 1320 bp in size, respectively. The predicted molecular weight of *SLC13A5* was approximately 61183.7 kDa and the theoretical isoelectric point was 7.45. Among 20 amino acids encoded by the *SLC13A5* protein, Leu was in the highest proportion and accounted for 13.4% of amino acids; His had the lowest proportion (1.2%). Analysis of the primary protein structure revealed that *SLC13A5* comprised a single peptide, eight putative transmembrane domains, and a low complexity region with 12 amino acids (Figure 2) that included 18 phosphorylation sites (Ser 6, Thr 9, and Tyr 3).

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TTTCTTTTTCCGGGAGTGACATCCGTCATATAGAACAAATAGTGTACTCAGCTTTCT 60
CTCCCGTTCGTCTATCGATGGGTTCGGTCTGATAGGCACTATCCGTCCGCGCGCGGAAA 120
CTCCGATCATCGCACTCTTCCCTATACCATCAATGCTTCTCGGACGGCATATCCGC 180
TTCAATGCCGCCAGGAGTCTTCTGGCTATCAAGCCCGGCTACTTTACCGGAAG 240
CGTAGGAACCCAGCAGTGGGTCTCTGGCATCCTATGCCTTTACTTTCTAGCGGAGGCGC 300
GGCCGATGATCGGCTGCTCGCGGTGGAGTCCCTCAGATGAATCCCTTTTACCTGT 360
CTTCTGTGACTCTCCGCTCTCCGCCAGGTTCCCTCCGCGC GCTCGGCACTGAGC 420
TATGCTCCCAAGTTAAGTCTTCTGTAICTGTTGTCACCCCGGTACTGCTGCTGCCA 480
TCAATCTGATGCGCCCAAGTTGTGAGGTGTGCCTACGTCATCACTGTCAAGGCC 540
GTTTACTGGTGCACAGAAGTCATCCCTCTGGCTGTCACCGCTCTCATGCCTGCTTGTCT 600
TTCCCACTTTCAGGATATAGGACTCGAAGCAGGTGTGCTCCAGTACATGAAGGACAC 660
AACATGCTGTCTCCGGGCGCCCTACGTTGGCCTGGCTGGAGCACTGGAACCTGGCAC 720
AAGAGGATGCGCTGCGCACGCTTCTGGTGGGGCCAGCCTGCACAGCTGATGCTG 780
KRIARLR TLLWV GAK P A D I M 126
GGCTTATGGGCGTACCGCCCTTCTCTCATGTGGATCAGCAACACGGCCACCGGCCC 840
GFMGV T A F L S M W I S N T A T T A 146
ATGATGGTGGCCATGTGGAGGCGTGTCTGCAGCAGATGGAGGCCACGAGCACAGCCAC 900
M M V P I V E A V L Q Q M E A T S T A T 166
GAGGCCAGCCTTGGGGCCCTGGAGCTGCGCAGACAAGGGCAAGGCTGGCGAGCTGCC 960
EASLG ALELE LADK GKAGELPG 186
AACCAAGCAAATTCGAAGGCCCTGCCATGAGGAAAGCAGGAGTTCTATGAGAGGAAGAAC 1020
NQA NFE GP A M R K Q E V H E R K N 206
GTATGCAAGGCCATGACCTGTGGCTGTGCTACGCAGCCAGCATGGAGGCCACTGCCACC 1080
VCKAM T L C V Y A A S I G G T A 226
CTGACCGGACAGGACCCAACTGGTACTCTGGGCCAGATGCAAGAAATGTTCTCTGAC 1140
T D T G P N Y V L L G Q M Q E L F P D 246
AGCAAAAGACATCGTGAACCTTCGCTCTTGTTGGATTTGCCTCCCTAACATGCTGATC 1200
S K D I V N A S W F G F A F P N M L 266
ATGCTGCTGCTCTCTGGCTGTGGCTACGGTTTATATATAGAGATTCAATATTAAAGAG 1260
M L L L S W L W L R F I Y M R F N I K E 286
TCCTGGGCTCGGGCTGGAGGGAAGAGTGAAGAAAAGGCTGCGCGAGAGGTGCTATGG 1320
S W G C G L E R K S E E K A A R E V L W 306
GTGGAGTACAGGAAGCTGGGACCTCTCTCTCGCTGAGGTCAACGTCCTGCTCTGCTTC 1380
V E Y R K L Q P L S F A E V N V L L C 326
TTCTGCTGTGGCTGTGGTCTCCCGGACCCCGGCTTCAATGCTGGCTGGCAGTCC 1440
L L L V G L W F S R D P G F M P G W Q S 346
ACACCTGGGTGGAGGGCAAGCAAGTTACACCTCTGATGCCACTGTGGCCATCTTGTG 1500
I T W V E G K S Y T S D A V A T T V 366
GCCTGCTGCTCTCATCATGCCCTTGTGAGAAGCCTAAGTCAACTTCTGCAGCCAGACT 1560
A C L L T J M P S E K P K F N F C S Q T 386
GAGGAAGAAGGAAAACCTCCATTTATCCCTCCCGCTGCTGAATGGAAAGTGGTCCAG 1620
E E E R K T P F Y P P P L L N W K V V Q 406
GAGAAAGTACCCTGGGGCAAGCTGTGCTCTCGGGGGCGGCTTGTCTGGCTAAAGGA 1680
E K V P W G I V L L L G G G F A L A K G 426
TGCAGGACTCGGGCTGTCAAGATGGATGGGAAAGCAGATGGAGCCCTTGCATGCTGTG 1740
C E D S G L S E W M G K Q M E P L H A V 446
GCCCCAGCAGCCATACCTTGTCTTGTCTCGCTCATCGCTGTGCTCACTGAGTGCACA 1800
A P A A I T L L I L S S L I A V L T E C T 466
AGCAACGTGGCCACCCTACCCTGTCTGCCCACCTTTGCTCCAGTCAAGTCCATT 1860
S N V A T T T L F L P I F A S M S R S I 486
GGCTCAACCCGCTGTACGTCATGATCCCTGTACCCCTGAGCTCATGCTTTGGCTTCATG 1920
G N P L Y V M I P C T L S S S F A F 506
CTGCCGTGGCCACCACCTAATGCCATGTGTTTTCTACGGACACCTCAAGGTTTC 1980
P V A T P P N A I V F S Y G H L K V S 526
GACATGGTAACTCAGCTGCTTTTATCAATCCTATCAGCTCTCTTGGTTTTGTATGGGG 2040
D M V T Q L L L L E N P I S S L A F V M G 546
GATGCCCATTTGTTAATGACCTG T T C A A C A G T T T G G G A A T C C A A A A A A A A 2110
D A P F V N D L * 554
AAAAAAAAAAAAAAAAAAAA 2118
    
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**Figure 1.** cDNA and putative amino acid sequences of porcine *SLC13A5* gene and open reading frame of the *SLC13A5* nucleotide sequence are underlined; asterisk indicates the stop codon, and start codon and stop codon are shaded with red; the signal peptide is shaded with purple; one low-complexity region is shaded with yellow; eight putative transmembrane domains is shaded with green.



**Figure 2.** *SLC13A5* conservative structure domain analysis in pig.

### Deduced protein sequence analysis, putative secondary and tertiary structures, and phylogenetic tree analysis

A comparison of porcine *SLC13A5* with sequences from 13 other animal species was carried out using the DNAMAN software; results are shown in Figure 3. Secondary and tertiary

protein structure predictions are presented in, Figures 4 and 5 respectively. Based on phylogenetic tree analysis, porcine *SLC13A5* sequence clustered with those of the sheep and the cattle, followed by *SLC13A5* sequence from the Rhesus, the African clawed frog, the wild pigeon, and the ground tid (Figure 6).

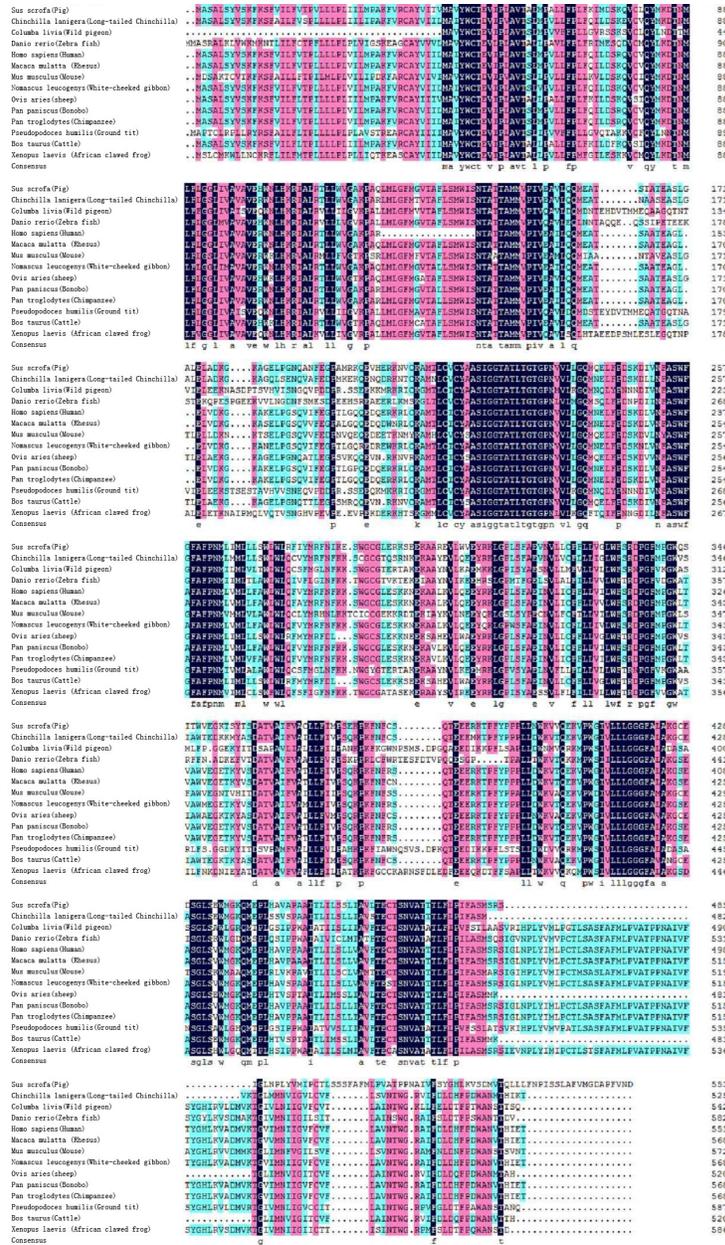


Figure 3. Alignment of amino acid sequences of the *SLC13A5* gene in fourteen different breeds.



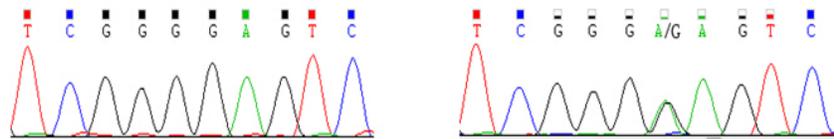
### Polymorphism detection and allele frequency

In this study, an SNP site was detected in intron 12 (Table 1). This mutation was reported for *Sus scrofa* (GenBank accession No. rs319369248) and its gene frequency was not reported. A nucleotide substitution of A by G was observed at the 251 position of the 815-bp fragment amplified with *Bsu361* restriction endonuclease (Figure 7). Three genotypes, AA, AG and GG, were generated (Figure 8). Allele G was predominant in Ningxiang and Shaziling pigs, while allele A was predominant in European and Taoyuan black pigs (Table 2).

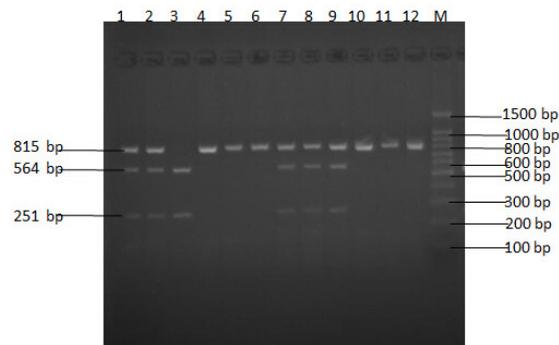
**Table 1.** Primer sequences used in this study.

Primer	Primer sequence	Size (bp)	Annealing temperature (°C)
<i>SLC13A5</i> -SNP1	F: CAGGCGTTGGGCTAAATG R: TGATGAGTGGCAGCAGCAGTA	997	60.0°
<i>SLC13A5</i> -SNP2	F: GCTTCTTCTGCTGGTCCG R: CAGTGGCATCAGAGGTGTA	943	60.0°
<i>SLC13A5</i> -SNP3	F: TCTTTGAGGGTGTATCTTT R: TCCTTCTATGGCACTATGT	893	54.0°
<i>SLC13A5</i> -SNP4	F: GCCACCACTACCCTGTTC R: TGCTTTCTTCTCAATCCC	711	59.5°
<i>SLC13A5</i> -SNP5	F: CTCATTCTGCGTCTTATTC R: GCTGTGGGTGGTGCATT	815	60.0°
<i>SLC13A5</i> -qPCR	F: TGACAGCAAAGACATCGTGAA R: TTAGGGAAGGCAAATCCAAAC	54	59.0°
GAPDH	F: ATTTGGCTACAGCAACAGGGT R: AAGTCAGGAGATGCTCGGTG	172	59.0°
5'-RACE	GSP5: TGACGTAGGCACACCTGACAA AUAP: GGCCACGCGTCGACTAGTAC	557	55.0°
3'-RACE	GSP3: GCGGTCACGGCCTTTCTCTCCATGT UPM : CTAATACGACTCACTATAGGGC	1320	65.0°

GSP = gene-specific primer; UPM and AUAP = universal and abridged universal amplification primers.



**Figure 7.** Sequencing maps of SNP for the *SLC13A5* gene.



**Figure 8.** Electrophoresis patterns obtained from digestion with *Bsu361* restriction enzyme. Lanes 1, 2, 7, 8, 9 = AG genotype; lane 3 = GG genotype; lanes 4, 5, 6, 10, 11, 12 = AA genotype; lane M = 100-bp DNA Ladder marker.

**Table 2.** Genotypic distributions, allelic frequencies of the *SLC13A5* gene of *Bsu361*-PCR-RFLP genotype in five pig breeds.

Breed	Sample size	Genotype frequency			Allele frequency	
		AA	AG	GG	A	G
Yorkshire	330	160	146	24	0.7061	0.2939
Daweizi	80	17	34	29	0.4250	0.5750
Shaziling	57	5	24	28	0.2982	0.7018
Ningxiang	65	5	6	54	0.1231	0.8769
Taoyuan black	112	71	37	4	0.7991	0.2009

Allele frequency was accurate to fourth decimal point.

### Relationship between SNP and pig growth traits

Least square means of data were calculated for the AA, AG, and GG genotypes by general linear model. The genotype was not significantly correlated with age at 100 kg and corrected back fat thickness ( $P > 0.05$ ; Table 3).

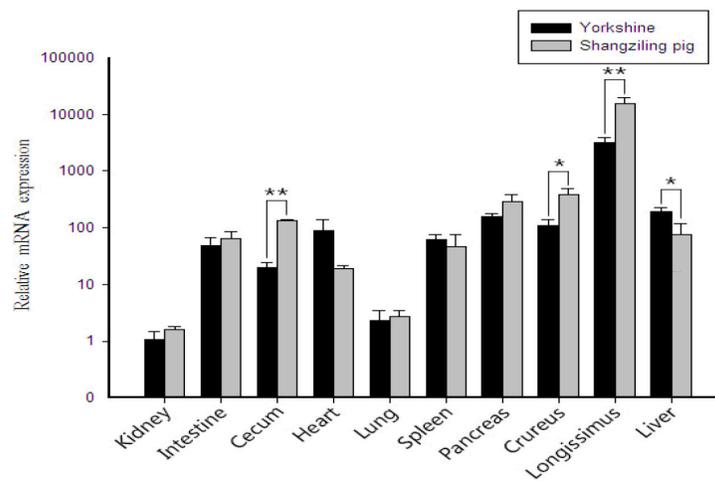
**Table 3.** Effect of three genotypes of the *SLC13A5* gene on growth trait in pigs.

Traits	Least square means $\pm$ SD		
	AA	AG	GG
Age at 100 kg (day)	170.0690 $\pm$ 12.5784(136)	170.3370 $\pm$ 11.2826(120)	172.2190 $\pm$ 9.6174(21)
Correction backfat thickness (CF) (mm)	12.1378 $\pm$ 3.1917(136)	12.9885 $\pm$ 3.3170(119)	13.0911 $\pm$ 3.929(22)

CF is calculated by the formula  $CF = 1.0981 'A / \{A + [B'(weight - 100)]\}$ , for male pigs A is 12.402 and B is 0.106530; for female pigs A is 13.706 and B is 0.11964.

### Tissue expression pattern of porcine *SLC13A5*

The expression pattern of the *SLC13A5* gene in ten different tissues of Shaziling pig and Yorkshire pig was analyzed by qPCR (Figure 9).

**Figure 9.** Expression profiles of the *SLC13A5* gene in ten different tissues of Shaziling and Yorkshire breeds. Bars indicate the standard deviation of the mean. \* $P < 0.05$ . \*\* $P < 0.01$ .

Expression levels of *SLC13A5* in cecum and longissimus dorsi muscle were significantly different (both  $P < 0.01$ ) in each of breeds, while significant in the liver and crureus muscle (both  $P < 0.05$ ). In each of pig breeds, *SLC13A5* expression levels in longissimus dorsi muscle differed significantly from those in the nine other tissues assessed ( $P < 0.05$ ). Levels of *SLC13A5* mRNA were high in the longissimus dorsi muscle, very low in kidney and lung, and moderate in other tissues.

## DISCUSSION

In this study, porcine *SLC13A5* coded 554 amino acids. The murine *SLC13A5* comprised 572 amino acids, and its sequence was highly analogous to human and rat *SLC13A5* (Inoue et al., 2002, 2004). Mouse *SLC13A5* was approximately 23 kb in length, and contained twelve exons. Phylogenetic tree analysis indicated that the cattle, the sheep, and the pig formed one cluster, whereas the bonobo, the chimpanzee, the human, the white-cheeked gibbon, and the Rhesus constituted another. Finally, they formed a cluster with the wild pigeon and the ground tit. The porcine gene clustered with that of other mammals. *SLC13A5* appears to be highly conserved during the process of species evolution.

The *SLC13A5* protein was found to be a soluble protein through hydrophilic analysis (Pajor, 2006; Shulman and Helfand, 2011). In our study, bioinformatic analysis showed that 1-38 amino acids of porcine *SLC13A5* existed in signal peptide, eight transmembrane regions were distributed among 45-67, 87-106, 121-140, 216-238, 253-275, 312-334, 354-373, and 488-510 amino acids; furthermore, there was a low-complexity region in 415-426 amino acids. We observed six serine (Ser), nine threonine (Thr), and three tyrosine (Tyr) sites, with a total of 18 potential phosphorylation sites.

In our study, porcine *SLC13A5* mainly expressed in the longissimus dorsi muscle, and expressed in moderate levels in liver, crureus, and pancreas, low levels in kidney. Markovich and Murer (2004) reported that *SLC13A5* expression was the highest in the gastrointestinal tract and epithelial cells of the kidney. It was expressed in normal human intestine (Weerachayaphorn and Pajor, 2008); however, mRNA of *SLC13A5* was found not to be expressed in normal human intestine by RT-PCR (Arai et al., 2012). Gopal et al. (2015) reported that *SLC13A5* also was predominantly expressed in the liver.

Our results showed that allele *G* was predominant in Ningxiang and Shaziling breeds, but allele *A* was predominant in European and Taoyuan black breeds. This SNP was not significantly correlated with age at 100 kg and corrected back fat thickness. The reason for this was the inadequate data. Also, *SLC13A5* maybe was mainly expressed in the meat, not in fatty tissues. Further study is needed, however, to verify whether this SNP is associated with IMF formation.

## CONCLUSIONS

In conclusion, we have characterized the *SLC13A5* gene. This gene expressed in a high level in longissimus dorsi muscle of 25-day-old piglets. Our research provides an expression and structural basis for further study on functions of the *SLC13A5* gene in pigs. Also, this study may provide the foundation for marker assistant selection (MAS) aimed at improving meat quality in pigs.

## Conflicts of interest

The authors declare no conflict of interest.

## ACKNOWLEDGMENTS

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