



Molecular characterization, tissue expression profile and SNP analysis of porcine *GLP2R*

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ABSTRACT. Glucagon-like peptide-2 receptor (*GLP2R*), a member of the G-protein-coupled receptor family, plays an important role in intramuscular fat formation. Little is known, however, about porcine *GLP2R*. In the present study, *GLP2R* was cloned, and its expression in pig muscle characterized. By rapid amplification of cDNA ends, gene sequence was obtained from Shaziling pigs. Full-length cDNA was 1868 bp, including an open reading frame 1665 bp in length, encoding 554 amino acids, and 203 bp at the 3' end. The *GLP2R* homology between porcine and other species was performed using bioinformatics techniques to construct a phylogenetic tree. Porcine *GLP2R* was most closely related to those from *Orcinus orca* and *Ovis aries*, and most distantly related to those from *Chrysemys picta*, *Taeniopygia guttata*, and *Falco peregrinus*. Real-time PCR analysis showed expression of porcine *GLP2R* in 10 different tissues from 25-day-old Yorkshire and Shaziling piglets, with expression levels being highest in the longissimus dorsi muscle and lowest in kidney. For each pig breed, expression level in longissimus dorsi muscle was highest among ten tissues ($P < 0.05$). Between the two breeds, *GLP2R* expression levels were significant in pancreas, the crureus and longissimus dorsi muscles ($P < 0.05$). A single SNP of porcine *GLP2R*, A343G, was identified, and

genotypes were determined by PCR-RFLP. This study provides an insight into the function of *GLP2R* in swine.

Key words: Pig; Expression profiles; Single nucleotide polymorphism; *GLP2R*; Cloning

INTRODUCTION

Glucagon-like peptide-2 (*GLP2*) is a recently-discovered intestinal epithelium specific growth factor that contributes to intestinal growth and development (Jiang et al., 2008; Hsieh et al., 2009), intestinal mucosal pathological damage repair (Wu et al., 2010), and intestinal barrier function (Moadebi et al., 2007; Yazbeck et al., 2009; Jiang et al., 2012). Additionally, *GLP2* is involved in the inhibition of intestinal cell apoptosis (Burrin et al., 2005); enhancement of intestine transport; and absorption of nutrients (Petersen et al., 2003; J de Heer et al., 2007; Han and Zou, 2010).

Glucagon-like peptide-2 receptor (*GLP2R*) is a member of the G-protein-coupled receptor family (Qiu and Chen, 2013; Mo et al., 2014) and is highly homologous to glucagon-like peptide-1 (*GLP1R*), glucagon (*GCG*), and glucose-dependent insulinotropic polypeptide (*GIP*) receptors (Park et al., 2013). As a specific binding receptor for *GLP2*, *GLP2R* also plays a regulatory role in intestinal mucosal cell proliferation, differentiation and repair (Taylor-Edwards et al., 2010). Luo et al. (2012) demonstrated that *GLP2R* played a role in porcine intramuscular fat, which was one of the major parameters influencing meat quality characteristics (Fernandez et al., 1999; Suzuki et al., 2005; Wood et al., 2008). Consumer demand for high quality meat is increasing (Gao and Zhao, 2009; Gjerlaug-Enger et al., 2010). However, over the past few decades, breeding programs of swine have focused on production of carcasses with high lean meat and extremely low fat, resulting in considerably decreased intramuscular fat (IMF) content (Cameron et al., 1990; Grindflek et al., 2001). Recently, there have been enormous endeavors to improve meat quality by enhancing IMF content. A number of genes have been reported to be associated with IMF of pork, such as *FAPB3*, *LEPR*, and *LDLR* (Li et al., 2011; Serao et al., 2011). It would therefore appear that an understanding of the molecular mechanisms of fat accumulation is important in improving IMF content of pork.

GLP2R is located on porcine chromosome 12, mouse chromosome 11, and human chromosome 17. To date, *GLP2R* cloning and expression has been achieved for rat, human and mouse (Shin et al., 2005); studies of porcine *GLP2R* are rarely reported. In the current study, porcine *GLP2R* cDNA was cloned using the rapid amplification cDNA ends (RACE) technique (Yeku and Frohman, 2011). Bioinformatics and phylogenetic tree analyses were performed based on porcine *GLP2R* amino acid sequence, and real-time quantitative PCR (Winer et al., 1999; Jin et al., 2013) was adopted to inspect *GLP2R* expression patterns in ten tissue types from two pig breeds. The purpose of the study is to lay a foundation for the study of *GLP2R* gene in the formation of IMF in the porcine.

MATERIAL AND METHODS

Sample collection

The ear tissue samples were from Ningxiang (N = 62), Shaziling (N = 25), Daweizi (N = 45), Taoyuan black (N = 74), and Yorkshire (N = 293). Three Shaziling and three Yorkshire pigs

are all aged 25 days for real-time quantitative PCR. Ten tissues from each breed were collected, including *longissimus dorsi*, crureus muscle, lung, liver, pancreas, intestine, cecum, heart, spleen, and kidney. Samples were immediately snap-frozen by liquid nitrogen and stored at -80°C until further processing. Pig genomic DNA was extracted from five different breeds, using the phenol/chloroform method.

RNA extraction, reverse transcription, cloning and sequencing

Total RNA was extracted from the *longissimus dorsi* of Shaziling pigs using the total RNA extraction kit in accordance with manufacturer instructions. The RNA quality and concentration were evaluated for each sample using a Nanodrop 2000 spectrophotometer. Total RNA was treated with *DNase I* and then first-strand cDNA was synthesized using the SUPERScript II RT Reverse Transcription System kit, following the manufacturer protocol (Invitrogen, USA). The conditions for PCR amplification were based on human *GLP2R* mRNA (GenBank No.: NM_004246) and were 94°C for 5 min; 32 cycles of 94°C for 20 s, 59°C for 25 s, and 72°C for 30 s; and 72°C for 5 min. Amplified products were purified on a gel, then cloned into pMD19-T vector (Takara, Japan) and sequenced.

Rapid amplification of cDNA ends (RACE)

To obtain full length *GLP2R* cDNA, two primer pairs were designed to amplify 5' and 3' ends of the gene, based on the coding sequence (CDS) obtained for porcine *GLP2R*. Using SMART™ RACE cDNA Amplification Kit, 5'-RACE and 3'-RACE PCR were performed according to the manufacturer protocol (BD Science, USA). Gene-specific primers (GSPs), universal primer (UPM), and abridged universal amplification primer (AUAP) were presented in Table 1. Amplified products were separated on an agarose 1.5% (w/v) gel. Following gel purification, products were cloned into the vector pMD19-T (Takara, Japan) and sequenced. Full-length cDNA sequence was assembled using DNASTar 7.1 software (Madison, Wisconsin, USA). Alignment of porcine cDNA with the sequence published in GenBank (National Center Biology Information, NCBI) was performed using the online software Basic Local Alignment Searching Tool (BLAST) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Table 1. Sequences of primers used in the study.

Primer	Nucleotide Sequence (5'–3')	Annealing temperature ($^{\circ}\text{C}$)	Size (bp)
GLP2R	F: ATGAGGCCGGAACGGAGCCG R: CTAGATCTCACTCCTCGAG	65	1665
3'-Race	3'445-1: CGGAGAGCAGCGAGGGCGACTTCAC UPM: CTAATACGACTCACTATAGGGC	65	420
GLP2R-SNP	F: CGGGAACCTCTGTTGTGC R: TGAAGCGGTGAGAATGGTA	61	746
Q-GLP2R	F: CATCCCTTTACTGGGTGTTCA R: AGAGTCAACTGAATGAAGAGTCGTA	59	102
S-GAPDH	F: ATTTGGCTACAGCAACAGGGT R: AAGTCAGGAGATGCTCGGTGT	59	172

Bioinformatic analysis

Physical and chemical properties of porcine *GLP2R* were analyzed using DNASTar 7.1 software. Nucleotide (nt) sequence analysis and amino acid (aa) sequence deduction were

performed using the Expert Protein Analysis System (<http://www.expasy.org/>). Protein domain features were predicted using the simple modular architecture tool (<http://smart.embl-heidelberg.de/>). Porcine *GLP2R* sequence was compared with sequence from 12 different species using DNAMAN V6 software, which was also utilized to align the aa sequences of *GLP2R*. A phylogenetic tree was constructed with the full-length cDNA sequences of previously published *GLP2R*, using the neighbor-joining (NJ) method with MEGA version 5.0 (Tamura et al., 2011).

Real-time quantitative PCR (qPCR) analysis

Expression of porcine *GLP2R* gene was evaluated by real-time quantitative PCR (qRT-PCR). Isolation of RNA was carried out using an RNA extraction kit, following the manufacturer instructions (Qiagen, Dalian, China). Total cDNA was generated using the QuantiTect Transcription Kit (Qiagen, Dalian, China). Real-time PCR was carried out in the Real-time PCR System (ABI) with SYBR Green primers using Premier 5.0 software (Table 1). Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was an internal control gene, and gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ comparative CT method. Amplification reactions were carried out in a final volume of 20 μ L comprising 10 μ L 2X qPCR Mix (ABI, USA); 1 μ L each primer (10 μ M); 1 μ L template cDNA; and 8 μ L ddH₂O. The PCR conditions were 95°C for 2 min; 40 cycles of 95°C for 30 s and 60°C for 40 s; and 72°C for 30 s. Using statistical software SAS10.0 for statistical analysis, significance differences of porcine *GLP2R* gene among different tissues and different breeds of pigs was analyzed (Figure 5). Porcine *GLP2R* mRNA expression levels were highest in *longissimus dorsi* muscle and expression levels differed significantly ($P < 0.05$) between tissues. Kidney and lung had the lowest expression and differences were also significant ($P < 0.05$) with other tissues.

SNP identification and allele frequency analysis

Primers GLP2R-SNP (Table 1) were used for detecting SNP in five different breeds. Genotyping was performed by PCR-RFLP technique using *BstE* II restriction enzyme (Fermentas, Beijing, China). The PCR products were digested by incubation at 37°C for 10 h in 20 μ L reaction mixture containing 9.8 μ L PCR products; 8 μ L nuclease-free water; 2 μ L 10X Buffer Tango; and 0.2 μ L 10 U *BstE* II. Restriction fragments were analyzed on a 2.0% agarose (w/v) gel.

RESULTS

Sequence analysis and comparison of amino acid sequence homology between porcine and other mammalian *GLP2R*

Assembly of the middle segment and 3'-RACE sequence resulted in a 1868 bp length cDNA sequence, which was submitted to GenBank (accession No. KF636493). The sequence comprised 203 bp of a 3'-untranslated region (UTR) as well as 1665 bp of an open reading frame (ORF) encoding 554 aa (Figure 1). Analysis of the ORF sequence demonstrated that the encoded protein had an approximate molecular weight of 62.96 kDa, and a theoretical isoelectric point PI of 9.279 (slightly alkaline). Results of Smart prediction demonstrated that along porcine *GLP2R*, aa residues 92 to 168 represented a hormone receptor (HormR) domain, while aa residues 176 to 433 represented PFAM: 7tm_2 domain (Figure 2). In addition, three pieces of low complexity sequence were noted at aa residues. The aa 188-204 was a low complexity for

SLSTASLFLALTL LLLCL sequence; aa 334-345 sequence was NNRNKNIWWIIR; and 256-371 sequence was FFIFLKILKLLISKLK.

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ATCAGGCCGGAAACGGAGCCGGGCGGGGCCCGGGAGGAAGCGAGCGGGGCTCTGCCTGAAGCC 63
M R P E R S R A G P G R K R A G L L P E A 21
CACGGGCGCCCCGGGCATCCCTGCCGCCGGGGGCCAGGCCCTCCCTCCCGAGGAAG 126
H G L P A G I P A A W G A R P L P S P R K 42
CGTGCTCCCGGACTCCTGTGAGCCCTTCATGGCCCTGCTGTTTCTGGTTCCATCAAGCAA 189
R A P R T P V R P F M A L L F L V S I K Q 63
GTTACAGGCTCTCTCCTTGAGGAGACAACACGGAAAGTGGGCTCAGTACAAAGAGAAGTGTCTG 252
V T G S L L E E T T R K W A Q Y K E K C L 84
AAAGACTTACTCAAGGAACTTCCGACACAGTGTGTACGGGACCTTCGATCGATATATATGC 315
K D L L K E T E D T V C N G T E D R Y I G 105
TGGCCCTCATTCCTCGCCGAAACGTTTCTGTCCCTGCCCTCATACTTACCTTGGTGGCGT 378
W P H S S A G N V S V P C F S Y L P W W R 126
GAAGAGAACTCTGGAAGGCCTACCATACTGCCTGCCTCGGGCTCCGTCAGACGCTGCAG 441
E E N S G R A Y R Y C L P R G S W Q T L Q 147
AACTCCACGGATGTTGGCAGGACGACTCCAGTGCTCCGAGGACCACAGCTCAAACAAAAT 504
N S T D V W O D D S Q C S E D H S F K Q N 168
GTGGAGCACCACGCCTTGTGTGCAGCTGCAGTGTGTACACGGTGGGCTACTCCCTC 567
V E H H H A L L S T L Q L L Y T V G Y S L 189
TCCACCGCTCCCTCTTCTGGCTCGACCTCCTCTGTGTCTTCGAAAACGCCACTGCACA 630
S T A S L F L A L T L L L C L R K L H C T 210
CGCAACTACATCCACATGAACCTGTTCCTCTTTTCACTCCGAAAGCCGTGGCCGTGCTGGCG 693
R N Y I H M N L F A S F I L K A V A V L A 231
AAGGACGTCATCTTTGACAGCTCTTACTCCAAGAGGCCGACAGTGAGAAAGCGTGGCTGTCC 756
K D V I F D S S Y S K R P D S E K A W L S 252
TACGTGTCCGAGATGTCGCCCTCCTGCCGACGGCCCAAGTCTCTCCTGCATCTACTCGTGGGC 819
Y V S E M S P S C R T A Q V L L H Y F V G 273
GCCAATTACTCGTGGCTGCTGGTAGAGGGCTTTACCTCCACACGCTGCTGGAGCCATCGCG 882
A N Y S W L L V E G L Y L H T L L E P I A 294
CTCTCTGAAAGCGCCTGTGGCCAGATACCTGCTGGTGGGTGGGCCTTCCCGGTGCTCTTT 945
L S E R R L W P R Y L L V G W A F P V L F 315
GTTGTCCCTGGAGCGTGGCCCGTGCAAAGCTGGAGAACAAGGGTGTGGGTAAACAACCGG 1008
V V P W S V A R A K L E N T G C W V N N R 336
AATAAGAATATCTGGTGGATCATCCGAGGACCCATGATGCTTTCGCTAATGGTCAACTTTTC 1071
N K N I W W I I R G P M M L C V M V N F F 357
ATCTTCCGAAAATCTCAAGCTTCTCATTCAAAAGCTCAAAGCTCATCAGATGTGCTTCAGA 1134
I F L K I L K L L I S K L K A H Q M C F R 378
GATTATAAATACAGGCTGGCAAAGCTCCACGCTGGTCCATCCCTTTACTGGGTGTCACGAG 1197
D Y K Y R L A K S T L V L I P L L G V H E 399
ATCGTCTTCGCTTTTCGTACGGACGATCAAGTGAAGGATTTGCGAAACTGTACGACTCTTC 1260
I V F A F V T D D Q V E G F A K L V R L E 420
ATTGAGTGTGACTCTGAGCTCCTTCCATGGACTGCTCGTGGCTTTCAGTATTGCTTTGCCAAC 1323
I Q L T L S S F H G L L V A L Q Y C F A N 441
GGAGAGTGAAGGCCGAGCTGC GGAAGCAGTGGGCCCGCTTCTTGCTAGCCACTACTCGGGC 1386
G E V K A E L R K Q W A R F L L A H Y S G 462
TGCAGAGCCTGGGTCTGGAGAAGAAGCTCCGATTCCGGGAAAATGTCACAGAAGGTCCCG 1449
C R A W V L E K N F R F L G K C P K K V P 483
GAGGGACTGGCACCAGGACACTGCAGAAGGCGCTGCCCTCGTCTGGCCGGGCGCAGTCCGCTG 1512
E G T G T G T L Q K A L P S S G R G Q S L 504
CACTGACCGCAGATGGGCTGGGGCAGGCAGAAGCCCTGCCCGCCATGGCCACCAGCCTGG 1575
H L T A D G L G Q A E A L P R H G H P A W 525
CCCAGGGCAGCAGCCTGTCGGAGAGCAGCGAGGGCGACTTCACCCTGGTCCACACCATGGAG 1638
P R G S S L S E S S E G D F T L V H T M E 546
GAGATTCGAGGAGAGTGAATC TAGACGGGGCCACCGCGCCTCTGCTCTCTGGGGACT 701
E I L E E S E I ★ 554
CCGGAGCCGGCCAGCCTGTGGTACGGGGTGCCAAGGCAGCACTCCTCCACGCCCCCTCCC 1764
ACTCCAACAGACATCTGTTTCCTGGAGGCTTTCTCTGCTCTGTATGACAGTGGCTTCATAAA 827
CACTCATCGCTTCCCAAAAAAAAAAAAAAAAAAAAAAAAAA 868

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Figure 1. Nucleotide and amino acid sequence of porcine *GLP2R* Open reading frame (ORF) of *GLP2R* nucleotide sequence is underlined. The stop codon is marked with a star, and start and stop codons are shaded in red. The HormR domain is shaded with green and PFAM: 7tm_2 domain is shaded with blue.

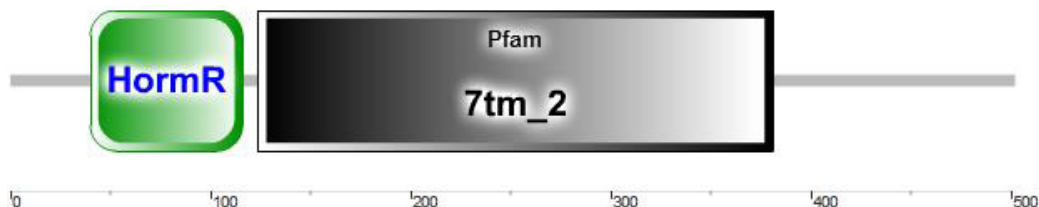


Figure 2. *GLP2R* conservative structure domain analysis.

Nucleotide sequence homology was compared between *GLP2R* from pig and 12 other species from the NCBI database, such as (*Taeniopygia guttata* (Zebra finch), *Trichechus manatus latirostris* (Florida manatee), *Orcinus orca* (Orca), *Felis catus* (Domestic cat), *Ovis aries* (Sheep), *Mus musculus* (mouse), *Dasybus novemcinctus* (Peba), *Tursiops truncatus* (Atlantic bottlenose dolphin), *Homo sapiens* (human), *Chrysemys picta bellii* (painted turtle), *Falco peregrinus* (peregrine), *Equus caballus* (horse). Results were shown in Table 2. Pig *GLP2R* nt sequence was most homologous to *GLP2R* of *Orcinus orca* (87.37%), and least homologous to *GLP2R* of *Chrysemys picta bellii* (60.25%). Results of homology analysis of deduced aa sequence were similar to those of nt analysis (Figure 3).

Table 2. GenBank version of *GLP2R* nucleotide sequence in different species and levels of homology.

Species	GenBank Version	Nucleotide Homology (%)	Amino Acid Homology (%)
<i>Taeniopygia guttata</i>	XM_002187228.2	60.95	54.80
<i>Trichechus manatus latirostris</i>	XM_004375982.1	82.27	77.12
<i>Orcinus orca</i>	XM_004266843.1	87.37	83.57
<i>Felis catus</i>	XM_003996288.1	82.24	79.54
<i>Ovis aries</i>	XM_004013306.1	85.89	81.41
<i>Mus musculus</i>	NM_175681.3	77.52	73.40
<i>Dasybus novemcinctus</i>	XM_004460224.1	76.58	64.57
<i>Tursiops truncatus</i>	XM_004313506.1	73.66	64.08
<i>Homo sapiens</i>	NM_004246.1	82.06	76.71
<i>Chrysemys picta bellii</i>	XM_005305108.1	60.25	56.39
<i>Falco peregrinus</i>	XM_005243602.1	59.79	56.29
<i>Equus caballus</i>	XM_001503249.2	84.63	80.51

Phylogenetic analysis

A phylogenetic tree was constructed from multiple alignments of the deduced aa sequences of porcine *GLP2R*, with the corresponding isoform sequences of other animals (Figure 4). Pigs had the closest genetic relationships to *Orcinus orca* and *Ovis aries*, and more distant genetic relationships to *Chrysemys picta bellii*, *Falco peregrinus*, and *Taeniopygia guttata*.

Tissue expression pattern of porcine *GLP2R*

Results are summarized in Figure 5. A contrario sensus analysis showed that expression levels in *longissimus dorsi* muscle differed significantly between the two pig breeds ($P < 0.01$) as did expression levels in pancreas and crureus muscle (both $P < 0.05$), while the differences in small intestine, cecum, heart, kidney, liver, lung, spleen was not significant between the pig breeds ($P > 0.05$).

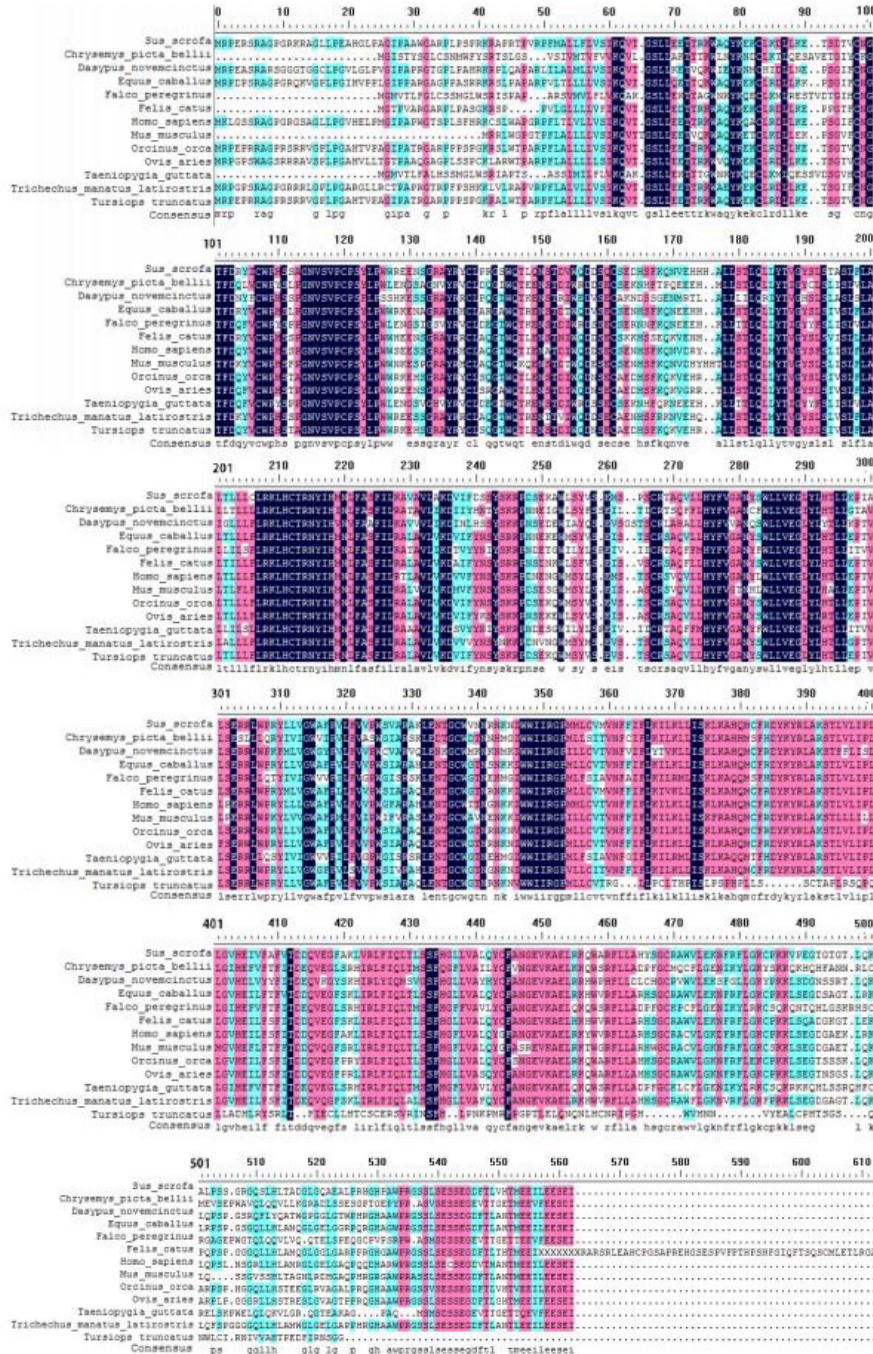


Figure 3. Amino acid sequence alignment of the *GLP2R* gene from different species. Levels of amino acid sequence similarity to *GLP2R* from different species are classified as follows: high (navy blue shading), intermediate (pink shading), and low (blue-green).

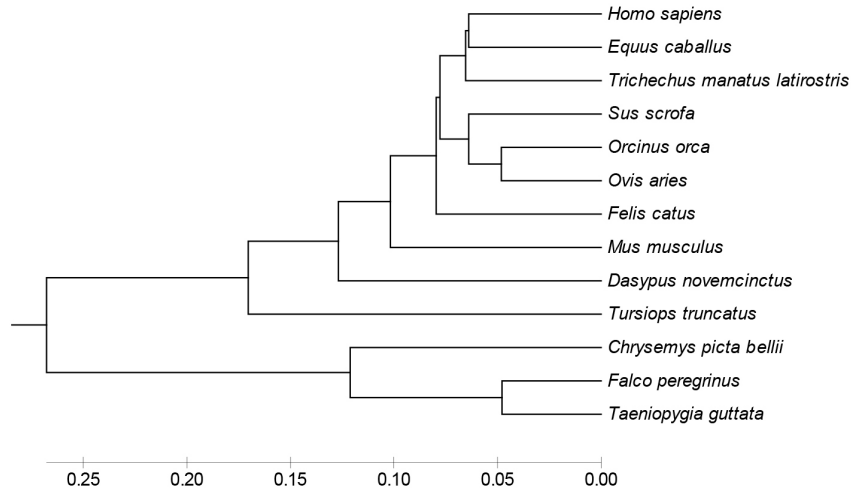


Figure 4. Phylogenetic tree based on amino acid sequences of *GLP2R*.

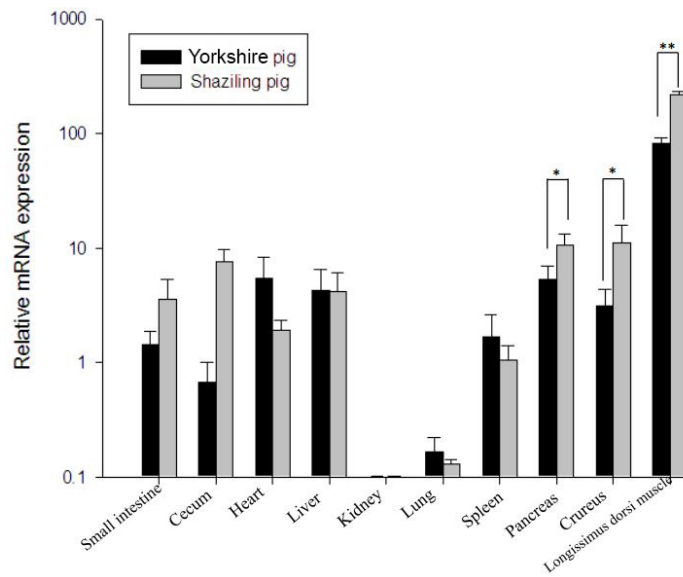


Figure 5. Expression of pig *GLP2R* mRNA by tissue type and pig breed ** is $P < 0.05$; *** is $P < 0.01$.

Polymorphism detection and allele frequency

A polymorphism was detected using the primer *GLP2R*-SNP. There was a nt substitution from A to G at the position 243 of the amplified fragment (Figure 6). Three genotypes for the polymorphism, GG, GA and AA, were generated. The results showed that allele G was predominant in Chinese native pig breeds (Ningxiang, Shaziling, Daweizi and Taoyuan Black breeds), while A was predominant in Yorkshire (Table 3).

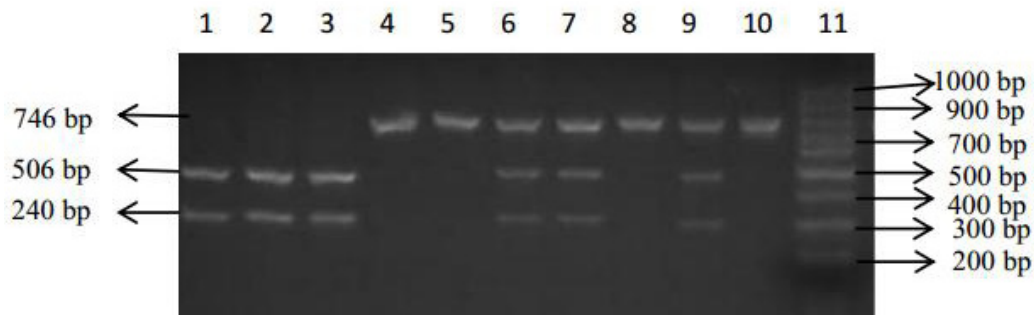


Figure 6. The electrophoresis patterns obtained digestion with *BstEII* endonuclease. Lanes 1-3, GG genotype; Lanes 3, 4, 8, and 10, AA genotype; Lanes 6, 7, and 9, AG genotype; M: 100 bp DNA Ladder Marker.

Table 3. Genotype distributions and allele frequencies of *GLP2R BstEII* genotype in five pig breeds.

Breed	Sample size	Genotype frequency			Allele frequency	
		GG	AG	AA	G	A
Ningxiang	62	62	0	0	1.0000	0.0000
Shaziling	25	3	21	1	0.5400	0.4600
Daweizi	45	45	0	0	1.0000	0.0000
Taoyuan black	74	51	8	15	0.7432	0.2568
Yorkshire	293	41	127	125	0.3567	0.6433

DISCUSSION

Protein phosphorylation, one of the most common and important means of protein modification after translation, was a common mechanism for life activity regulation that played an important role in the process of cellular signal transduction (Liang et al., 2012). Sixteen serine phosphorylation sites, 5 threonine phosphorylation sites and 2 tyrosine phosphorylation sites had been found in porcine *GLP2R*. These sites may played important roles in signal transduction of somatic cell and cell growth, differentiation, and other physiological processes.

Phylogenetic analysis of porcine *GLP2R* gene showed that pigs were close relatives to other mammals (such as *Orcinus orca* and *Ovis aries*) and relatively distant to reptiles (*Chrysemys picta bellii*) and birds (*Falco peregrinus*, *Taeniopygia guttata*). This result conformed to the general law of evolution. Taken together, these results suggested that the *GLP2R* gene was strongly conserved in the long-term evolution process, and had different species specificity. This suggested that it could play an important role in the process of biological growth and development.

We demonstrated that porcine *GLP2R* gene was expressed in 10 different tissues taken from the two pig breeds studied, and that expression levels were highest in *longissimus dorsi* muscle. In a previous study, porcine *GLP2R* expression levels were found to be significantly related to IMF, based on genome-wide association analysis (Luo et al., 2012).

Orskov et al. (2005) reported that in the digestive tract of non-ruminants, *GLP2R* mRNA was mainly expressed in the proximal small intestine, in particular in jejunum. Park et al. (2013) reported that cattle *GLP2R* mRNA was mainly expressed in the small intestine and colon tissue. In a study of mice, expression of *GLP2R* gene was detected in the duodenum, jejunum, ileum, and colon but not in the liver, lung, and kidney (Han et al., 2009). Differences between these studies may be due to such factors as animal age, as well as the different test methods used.

Petersen et al. (2003) observed that *GLP2R* mRNA expression levels in small intestine gradually declined with increasing animal age; that is, the neonatal piglet *GLP2R* mRNA abundance was the highest, and then decreased significantly toward weaning age. In the current study, animals were 25 day old at sampling, and, with the exception of *longissimus dorsi* muscle, *GLP2R* mRNA expression levels were low overall. Age-related declines in expression levels may account for this observation.

RACE technique was adopted in the study to successfully clone porcine *GLP2R*, alongside bioinformatics methods to analyze the sequence and characterize its encoded protein. The results presented here laid the foundation for future studies to explore the functions of *GLP2R*. It is important for the meat industry to improve meat quality, in particular, the IMF content of pork. Most recent studies on IMF focused on the mechanism of lipogenesis (Morcuende et al., 2007; Zhao et al., 2009), and numerous candidate genes associated with IMF deposition had been identified (Cánovas et al., 2010; Serão et al., 2011; Tyra and Ropka-Molik, 2011). As mentioned, porcine *GLP2R* is significantly related to IMF in pork (Luo et al., 2012). In the current study, an SNP site was identified in intron 5, but further work is needed to confirm if this SNP is associated with IMF. These results raised the possibility of using molecular marker assistant (MAS) selection in porcine breeding.

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