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Characterization of porcine *GAS6* cDNA gene and its expression analysis in weaned piglets

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ABSTRACT. The growth arrest-specific 6 gene (*GAS6*) is a member of the family of plasma vitamin K-dependent proteins, which are able to bind to phospholipids using an N-terminal gamma-carboxyglutamic acid domain. A recent report has demonstrated that the *GAS6* gene can promote fat deposition and is associated with an increased number of fat cells in mice. In order to investigate whether *GAS6* expression is associated with meat quality in pigs, a 2382-bp cDNA sequence of the porcine *GAS6* gene (GenBank accession No. KC526197) was first obtained using rapid amplification of cDNA ends from porcine longissimus dorsi tissue. One *A*/*G* single nucleotide polymorphism anchored in exon 12 was genotyped using the marker PCR-RFLP-*Bgl*I, and the *G* allele was dominant in the pig breeds tested. Quantitative real-time polymerase chain reaction showed that the porcine *GAS6* gene was expressed in all tissues examined in weaned male Shaziling (SZL) and Yorkshire (YS) weanling piglets, and mRNA expression of *GAS6* in the longissimus dorsi tissue of SZL piglets was significantly higher than that in YS piglets ($P < 0.05$). The GAS6 protein was likely to be a membrane protein and was detected in longissimus dorsi tissue from SZL and YS piglets using immunohistochemistry, with an

abundant protein expression index (P > 0.05). The results imply that the *GAS6* gene can be considered a potential candidate for meat quality trait selection and fat deposition in pigs.

Key words: Pig; *GAS6*; SNP; Expression profile; Immunohistochemistry

INTRODUCTION

The growth arrest-specific 6 gene (*GAS6*), first cloned using cultured NIH 3T3 cells without plasma, encodes the GAS6 protein, which is a vitamin K-dependent growth factor found in blood (Manfioletti et al., 1993; Mark et al., 1996), and the TAM receptors (TYRO3, AXL, MER) belong to the Axl receptor protein-tyrosine kinase (RTK) family (Godowski et al., 1995; Nagata et al., 1996; Chen et al., 1997). It plays broad roles in cell survival and proliferation, cell adhesion and migration, the stability of coagulation, the release of inflammatory factors, and physiological and pathological reaction processes (Eng et al., 2008; Ganopolsky et al., 2008; Xiong et al., 2008; Anwar et al., 2009; Scutera et al., 2009; Uehara et al., 2009; Wu et al., 2011). Manfioletti et al. (1993) suggested that the GAS6 protein contained 678 amino acids, with 81% homology to mouse protein and 44% homology to the human anticoagulant S protein (S protein is a negative regulator of blood, regulating the vitamin K-dependent pathway). Using by fluorescence *in situ* hybridization (FISH) method, the *GAS6* gene has been located on *HSA*13q34 (*Homo sapiens*) and chromosome 8A1.1-1.2 (*Mus musculus*) (Colombo et al., 1992; Saccone et al., 1995), and *GAS6* has also been located on 16q12.5 in rats (*Rattus norvegicus*) (http://www.ncbi.nlm.nih.gov/gene/58935). Our previous study assigned the porcine *GAS6* gene to SSC11q11-17 using the somatic cell hybrid panel and the radiation hybrid panel (Ma et al., 2005).

Recent research has implicated *GAS6* in several cellular functions, including reversible cell growth arrest (Manfioletti et al., 1993), survival and proliferation (Goruppi et al., 1996), apoptosis, and cell adhesion (Nakano et al., 1997; Fridell et al., 1998). *GAS6* has been shown to be a potential growth factor for many cell types, including fibroblasts (Goruppi et al., 1996), mesangial cells (Yanagita et al., 2001), and adipose cells (Maquoi et al., 2005). Several reports have shown that *GAS6* is preferentially expressed during the clonal expansion of postconfluent 3T3-L1 pre-adipocytes (Shugart et al., 1995), and it is involved in the reversible growth arrest of confluent pre-adipocytes, and that the down-regulation of its expression is associated with adipogenesis (Maquoi et al., 2005). Furthermore, the *GAS6* gene also plays an important role in differentiation of epithelial cells in the hair follicle (Varnum et al., 1995). Despite the potential implications of *GAS6* in these different functions, it has been reported that *GAS6*-deficient mice develop normally and are apparently healthy (Angelillo-Scherrer et al., 2001). Eight single nucleotide polymorphisms (SNP) from the human *GAS6* gene were studied to investigate its correlation with stroke, with 110 healthy people as the control group (Munoz et al., 2004). Preliminary analysis of 5 SNPs from 188 stroke patients indicated that the genotype frequency of one SNP (*G*/*A*) was highly significantly different between the experimental and control groups (Munoz et al., 2004).

According to the above results, *GAS6* can be considered a functional candidate gene for meat quality owing to its characteristics in humans and mice. We detected an SNP in the porcine *GAS6* gene that is related to meat quality; however, association of the *GAS6* gene with pork quality had not been reported. This study aimed to present the isolation of cDNA and expression profile of the porcine *GAS6* gene and investigate its expression level in ten tissues extracted from two pig breeds. Additionally, one SNP in *GAS6* was detected in eight foreign and domestic pig breeds and

its association with meat quality traits was analyzed. Our results provide further reference to reveal the function of the porcine *GAS6* gene.

MATERIAL AND METHODS

Sample collection

Total RNA was extracted from these muscle samples using the total RNA extraction kit (Invitrogen, Karlsruhe, Germany) and was amplified using the rapid amplification of cDNA ends (RACE) technique to generate the *GAS6* cDNA sequence.

Ear samples from eight breeds of pig were collected, *Duroc* (DR; N = 76), Landrace (LD; N = 64), Yorkshire (YS; N = 582), Shaziling (SZL; N = 77), Daweizi (DWZ; N = 49), Taoyuan (TY; N = 69), Ningxiang (NX; N = 68), and Wuzhishan (WZS; N = 55), and genomic DNA separated from these ear samples was amplified and sequenced directly for the identification of SNPs.

Samples from the longissimus dorsi, crureus, lung, liver, pancreas, intestine, cecum, heart, spleen, and kidney were collected from three full-sib male weaned SZL piglets for each of SZL and three full-sib male weanling YS piglets provided by Hunan Xinwufeng Breeding Company in Pig, Xiangtan, Hunan Province, China, and the Resource Field of Xiangtan Shaziling pig in Hunan, respectively. Samples were immediately snap frozen in liquid nitrogen and stored at -80°C until used. Total RNA was extracted from the tissue samples using total RNA extraction kits (Takara, Shiga, Japan). *DNase*I treatment of total RNA was undertaken prior to first-strand cDNA synthesis. RACE technique was used to generate the *GAS6* cDNA sequence. Also the RNA was performed quantitative real-time PCR (qRT-PCR) for tissue expression analysis. *DNase*I treatment of total RNA was undertaken prior to first-strand cDNA synthesis.

The experiment was conducted following the guidelines of the animal Ethics Committee at Hunan Agricultural University, China. The slaughter and sampling procedures complied with the ‘‘Guidelines on Ethical Treatment of Experimental Animals’’ (2006) No. 398 set by the Ministry of Science and Technology, China.

Isolation of the cDNA sequence

Total RNA was treated with *DNase*I, and then reverse transcribed with Moloney Murine Leukemia virus reverse transcriptase (Promega, Madison, WI, USA). The human *GAS6* gene sequence (NM000820) was compared with all sequences in the expressed sequence tag (EST) database using the standard NCBI BLAST (http://www.ncbi.nlm.nih/gov/blast/), and the porcine ESTs that shared at least 80% identity to the corresponding human mRNA were selected to design gene specific primers using the Primer Premier 5.0 software (http://www.premierbiosoft.com/primerdesign/) (Table 1). PCR products were purified with the 3S Spin DNA agarose gel purification system (ShenergyBiocolor, Shanghai, China) and cloned into the pMD18-T vector (Takara, Shiga, Japan), then sequenced by BioSune Biotech (Shanghai, China).

SNP identification and genotyping

Primers for the *GAS6* gene used for SNP identification were designed using the Primer Premier 5.0 software (Table 1). The genotyping PCR was performed in a 20-μL reaction volume consisting of 100 ng genomic DNA, 10X buffer, 0.5 μM of each primer, 75 μM of each dNTP,

1.5 mM $MgCl_2$, and 0.5 U *Taq* DNA polymerase (Promega). The PCR cycling conditions were as follows: 5 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 59°C, 40 s at 72°C, and a final extension of 10 min at 72°C. PCR products were directly sequenced to identify SNPs, and Chromas Pro and DNAMAN 6.0 softwares were used to analyze the sequences. PCR product (4 µL) was digested overnight with 3 U *Bgl*I (Thermo Fisher Scientific, USA) at 37°C, and then size-separated on a 2.0% agarose gel stained with ethidium bromide.

Table 1. Primers used to perform PCR amplifications of pig tissues.

Primer purpose	Primer name	Primer sequence (5'-3')	Tm (°C)	Product size (bp)
Cloning	Mid-seq-F	CCGAGGGTGTCCTGTTCTT	58	604
	Mid-seq-R	AATGAAGGTGAGCACAGAGCC		
	3'-F1	ACCCGCCCGAGACACTGAAT	55	817
	3'-F2	CACCTACAAGCACAGCGACATCA		
	5'-R1	CGCTGTAGCAGGCACAGTGGAA	55	733
	5'-R1	CAGTAGAAGTTGCCCATGAGGTCTGG		
	AAP	GCCACGCGTCGACTAGTACG10		
	AUAP	GGCCACGCGTCGACTAGTAC		
SNP genotyping	Genomic-F	GAGTGCCAAGGAGCAGAAAT	59	516
	Genomic-R	CCCGCTAAGGTGTGTTTGTT		
Expression profile (qRT-PCR)	GAS6-F	CCGAGACACTGAATTTTG	55	82
	GAS6-R	ACTGAGGTTAGTCTGCTAM		
Internal control	GAPDH-F	GGGCATGAACCATGAGAAGT	60	233
	GAPDH-R	AAGCAGGGATGATGTTCTGG		

Expression profiles of *GAS6*

Primers for qRT-PCR were designed using the Primer Premier 5.0 software (Premier, Canada) (Table 1) and qRT-PCR was carried out in a Bio-Rad qRT-PCR System (Bio-Rad). The results were calculated using the relative quantification method with the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as the internal control gene. *GAS6* expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). qRT-PCR amplification were carried out in a final reaction volume of 20 µL comprising 10 µL 2X qPCR mix (Takara, Shiga, Japan), 10 µM each primer, 1 µL template cDNA, and 8 µL ddH_2O, with the following cycling conditions: 95°C for 2 min, followed by 40 cycles of 95°C for 10 s, 55°C for 40 s, and 72°C for 30 s.

Immunohistochemical experiment

Following immunohistochemical methods (Fregly and Graybiel, 1968; Nishiwaki et al., 2000), longissimus dorsi tissue samples were fixed in 4% paraformaldehyde, embedded in paraffin according to routine histological procedures, sectioned at a thickness of 4 µm, and dehydrated through an alcohol series. Samples were then incubated with monoclonal antibody “bs-7549R” (1:100), specific to the GAS6 protein, at 4°C overnight, and washed 3 times with phosphate-buffered saline (PBS) for 5 min. Then, the sections were incubated with biotinylated anti IgG (1:50) for 10 min at room temperature, washed 3 times with PBS for 5 min as before,

and stained with 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO, USA) at room temperature for 5 min. Before being mounted, the sections were rinsed in tap water and counterstained with hematoxylin.

Statistical analyses

The sequence of porcine *GAS6* cDNA was analyzed using the Smart search (http://smart.embl-heidelberg.de/) and Expasy search (http://www.expasy.org/tools/) programs. Gene expression levels in the two pig breeds were analyzed using the SAS 9.1 software (SAS Inst. Inc., Cary, NC, USA). Results are reported as means ± standard error. The χ^2 test was used to analyze the distribution of genotypes and to compare differences in genotype distribution.

RESULTS AND DISCUSSION

Cloning and characterization of *GAS6* cDNA

Analysis of the porcine *GAS6* cDNA sequence revealed that the full-length cDNA sequence was 2382 bp, containing an open reading frame (ORF) of 2028 bp, encoding a protein of 675 amino acids, with a calculated molecular mass of 74.4 kDa and an isoelectric point (pI) of 5.37. The 5'-untranslated region (UTR) was 36 bp and the 3'-UTR was 318 bp, with a terminator codon (TAG) and a putative polyadenylation signal (AATAAA) found 22 bp upstream of the poly(A) tail (Figure 1A). The cDNA sequence of *GAS6* was deposited in GenBank under accession No. KC526197. The deduced amino acid sequence of the ORF contained nine functional domains (Figure 1B), including two low-complexity domains, one gamma-carboxyglutamic acid-rich (GLA) domain, one epidermal growth factor (EGF) domain, three calcium-binding EGF domains, and two Laminin G (LamG) domains, similar to the structure of *GAS6* in human, mouse, and zebrafish (Manfioletti et al., 1993; Ohashi et al., 1995; Song et al., 2004), suggesting that the function of *GAS6* is conserved.

Sequence alignment analysis (Table 2) showed that the deduced amino acid sequence shared 50.2 to 88.3% homology with various species, including *Camelus dromedaries* (XM_010987649), *Bos taurus* (XM_002692001.3), *H. sapiens* (NM_000820.3), *M. musculus* (NM_019521.2), *R. norvegicus* (NM_057100.2), *Callithrix jacchus* (XM_009007398.1), *Loxodonta africana* (XM_010593595.1), *Meleagris gallopavo* (XM_010728605.1), *Xenopus (Silurana) tropicalis* (NM_001015965.2), *Esox lucius* (XM_010886742.1), *Larimichthys crocea* (XM_010735342.1), *Notothenia coriiceps* (XM_010786633.1), and *Danio rerio* (NM_199978.1), with the highest homology (88.3% identity) with *C. dromedaries GAS6*, and the lowest homology (50.2% identity) with *D. rerio*. Multiple alignments (Figure 2 and Table 2) revealed that many of the protein domains are highly conserved. Human (*H. sapiens*) and mouse (*M. musculus*) *GAS6* genes have been cloned and characterized previously, and *GAS6* gene knockout in mice can inhibit thrombosis, suggesting it may play an important role in vascular systems (Yanagita et al., 2002; Munoz et al., 2004; Angelillo-Scherrer et al., 2005). Our study is the first to clone and characterize the *GAS6* gene in porcine muscle. The data presented here provide a basis for future studies on the function of the porcine *GAS6* gene.

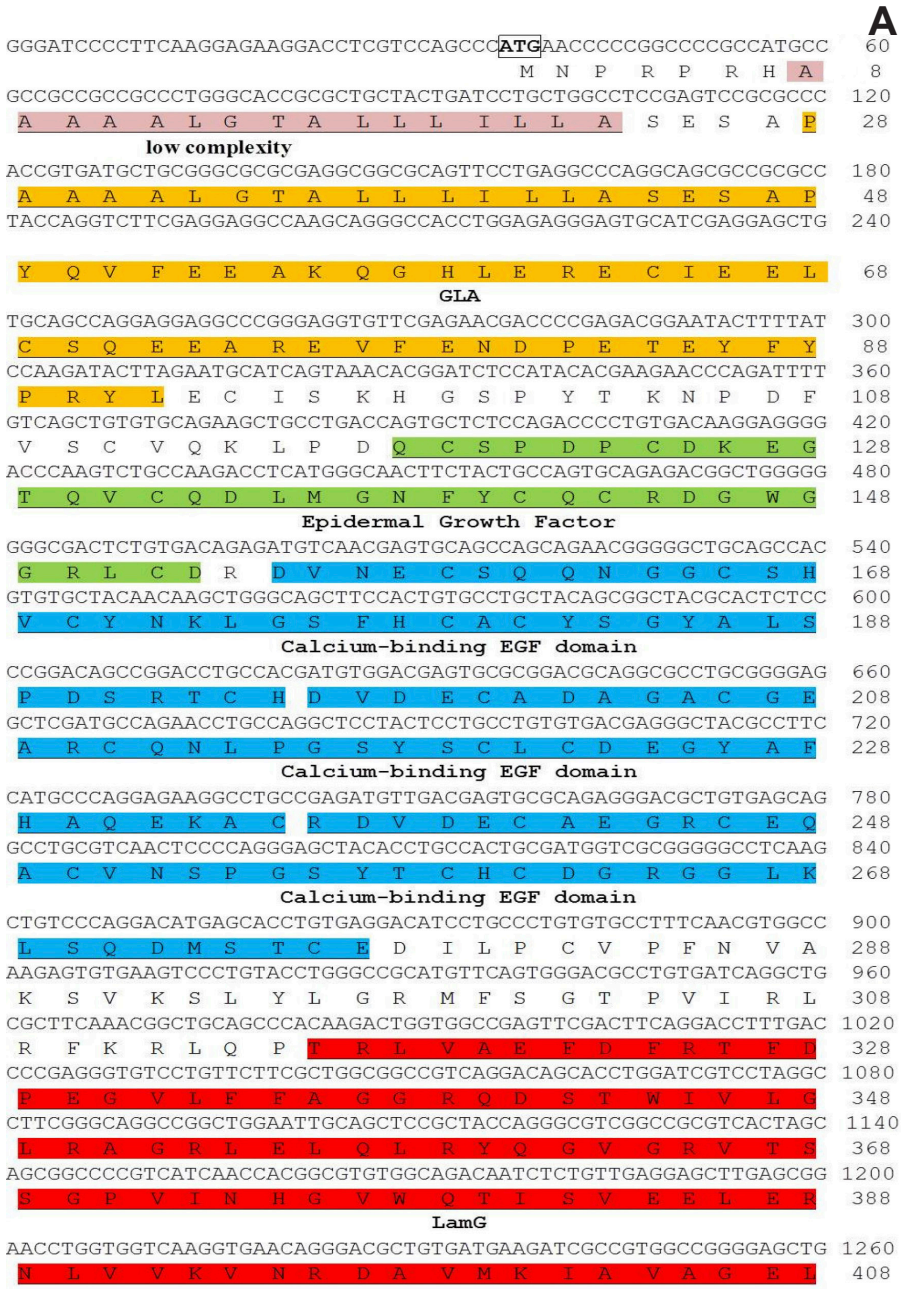

Figure 1. Nucleotide and predicted amino acid sequence of the porcine growth arrest-specific 6 (*GAS6*) gene. **A.** Bold letters in the box indicate the start codon (ATG), the stop codon (TAA), and the polyadenylation signal sequence (AATAAA). Domains are highlighted with colored backgrounds, including low-complexity domains, gamma-carboxyglutamic acid-rich (GLA) domain, epidermal growth factor (EGF) domain, calcium-binding EGF domains (EGF-CA), and Laminin G (LamG) domains. **B.** GAS6 conservative structure domain analysis.

Continued on next page

Figure 1. Continued.

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TTCCAGCTGGACAGGGGGCTGTACCATCTGAACCTGACTGTGGGGGGGATCCCCTTCAAG 1320
 F  Q  L  D  R  G  L  Y  H  L  N  L  T  V  G  G  I  P  F  K   428
GAGAAGGACCTCGTCCAGCCCATGAACCCCCGTCTCGACGGCTGCATGAGGAGCTGGAAC 1380
 E  K  D  L  V  Q  P  M  N  P  R  L  D  G  C  M  R  S  W  N   448
TGGCTGAATGGCGAGGACACCACCATCCAAGAAACGGTCCGCGTGAACGCGAAGATGCAG 1440
 W  L  N  G  E  D  T  T  I  Q  E  T  V  R  V  N  A  K  M  Q   468
TGCTTCTCCCTGACCGAGAGGGGCTCCTTCTTCCCCGGGACTGGGTTCGCCCTGTACAGC 1500
 C  F  S  L  T  E  R  G  S  F  F  P  G  T  G  F  A  L  Y  S   488
CTGGACTACGCTCGGACGTCGCCGGACATCGGGGCGGAGACCTTTTGGGAAGTAGAGGCT 1560
 L  D  Y  A  R  T  S  P  D  I  G  A  E  T  F  W  E  V  E  A   508
GTGGCTCGGATCCGCCCCGCCACTGACACGGGGGTGCTGCTCGCACTGGTTGGCGGGGAC 1620
 V  A  R  I  R  P  A  T  D  T  G  V  L  L  A  L  V  G  G  D   528
CAGGCCGTCGTGCTCTCCGTGGCCCTGGTCGACTACCACTCCACCAAGAAGCTCAAAAAG 1680
 Q  A  V  V  L  S  V  A  L  V  D  Y  H  S  T  K  K  L  K  K   548
CAGCTGGTCGTCCTGGCCGCGGAGAGCGTCACCCTGGCCCTGATGGAGATCAAGGTCTGT 1740
 Q  L  V  V  L  A  A  E  S  V  T  L  A  L  M  E  I  K  V  C   568
low complexity
GACGGCCAGGAGCACGTGGTGACCGTCTCTGTGAACAAGGACGAGGCCACCCTGGAGGTG 1800
 D  G  Q  E  H  V  V  T  V  S  V  N  K  D  E  A  T  L  E  V   588
GATGGCACCAGGGGCCAGCGTGACGTGAGCCCGGCAGGGCTGCAGGAGAGGCTGGTGCTC 1860
 D  G  T  R  G  Q  R  D  V  S  P  A  G  L  Q  E  R  L  V  L   608
                          LamG
CTGGGGAGGCACCTGCAGGGCTCTGTGCTCACCTTCATTGGGGGGCTGCCAGATGTGCCG 1920
 L  G  R  H  L  Q  G  S  V  L  T  F  I  G  G  L  P  D  V  P   628
GTGACCTCGGCCCCGGTCACTGCCTTTTACCGTGGCTGCATGACGCTGGAGGTCAACGGC 1980
 V  T  S  A  P  V  T  A  F  Y  R  G  C  M  T  L  E  V  N  G   648
AAGGCGCTGGACCTGGACGAGGCCACCTACAAGCACAGCGACATCACGGCCCACTCCTGC 2040
 K  A  L  D  L  D  E  A  T  Y  K  H  S  D  I  T  A  H  S  C   668
CCCCCCGTTGAGCCGGCCGCCTAGGCTCCCGCCACCAAGCCCTCCTCACGCTTTCTTCCC 2100
 P  P  V  E  P  A  A  .                                        675
AGACCAGGAAGGAGGGCGGGGGCACCAGCGCCCACCCGCCCGAGACACTGAATTTTGCCA 2160
GCTGAGGCCCCTCGCACCCCTCCGTCTGTAACATACGTGTAAATAGCAGACTAACCTCAG 2220
TGCTGCCCGAGCCTTCCTGGGGGCCTGGGCCGGCACGCGCCAGAGCGGACCCGGGGACAT 2280
AATTCTGTTATTTTTATTACCAGATGCTTCTTTCTGACCTAAAACTATGGGAAAATAAAG 2340
TATTTACGGAACCTTAAAAAAAAAAAAAAAAAAAAAAAAAA                     2382
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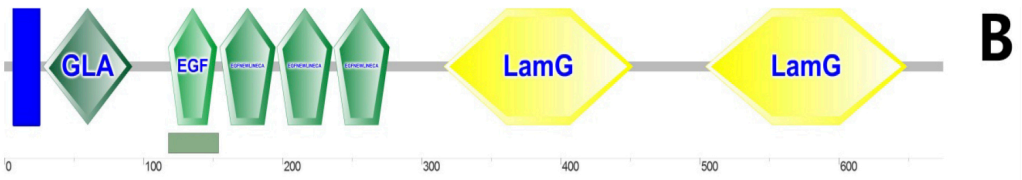

Figure 2. Multiple alignment of computationally complete and predicted growth arrest-specific 6 (*GAS6*) cDNA sequences (NCBI database) from various species with the *GAS6* sequence in porcine. Figure constructed using DNAMAN 6.0.

Table 2. Amino acid sequence identities of *Sus scrofa* growth arrest-specific 6 gene (*GAS6*) with *GAS6* in various species.

Species	Amino acid homology (%)													
S. scrofa	x													
B. taurus[b]	86.5	x												
C. jacchus[b]	78.8	78.6	x											
C. dromedarius[b]	88.3	86.5	80.2	x										
D. rerio[a]	50.2	50.2	50.2	50.7	x									
E. lucius[b]	53.1	53.2	52.2	53.1	67.2	x								
H. sapiens[a]	80.6	80.9	92.0	81.4	50.7	52.6	x							
L. crocea[b]	51.8	52.5	52.8	52.0	70.2	74.8	52.6	X						
L. africana[b]	79.5	77.2	79.7	81.7	49.9	53.1	79.3	51.7	x					
M. gallopavo[b]	65.8	65.3	64.1	66.1	51.1	53.7	65.4	52.3	67.8	x				
M. musculus[a]	80.4	78.6	80.1	81.1	51.5	52.5	80.9	51.8	80.7	64.3	x			
N. coriiceps[b]	51.4	51.9	51.9	51.4	69.6	75.6	51.7	87.5	50.6	51.4	51.1	x		
R. norvegicus[a]	79.8	78.3	81.0	80.8	51.0	52.8	81.8	52.3	81.3	64.1	93.9	51.5	x	
X. tropicalis[b]	61.8	62.3	60.3	62.2	49.9	52.2	61.9	51.7	64.2	65.5	63.4	51.3	63.6	x

[a]First published sequence. [b]Automated computational prediction by NCBI. *Camelus dromedaries* (*C. dromedarius*), *Bos taurus* (*B. taurus*), *Callithrix jacchus* (*C. jacchus*), *Loxodonta africana* (*L. africana*), *Meleagris gallopavo* (*M. gallopavo*), *Xenopus* (*Silurana*) *tropicalis* (*X. tropicalis*), *Esox lucius* (*E. lucius*), *Larimichthys crocea* (*L. crocea*), *Notothenia coriiceps* (*N. coriiceps*), and *Danio rerio* (*D. rerio*).

Polymorphism detection

One SNP, anchored in exon 12 of the *GAS6* gene, was identified. A genomic fragment of 516 bp (516 bp for allele *A*, and 332 and 184 bp for allele *G*) was amplified and subsequently genotyped using the marker PCR-RFLP-*Bgl*I (Figure 3). Three variants were identified in the *GAS6* gene. We genotyped the A2056G transition using RFLP-*Bgl*I in eight pig breeds (Table 3), and the genotype distributions were found to be in Hardy-Weinberg equilibrium ($P > 0.05$). The results indicated that allele *G* was predominant in all the breeds except for the mini-type indigenous breed (WZS, frequency of 0.4545). For the native breeds (DWZ, SZL, NX, and TY), the *G* allele was dominant, with the highest frequency (0.8636) in SZL. In the three foreign breeds (DR, LD, and YS), the *G* allele was also dominant, with the highest frequency (0.8779) observed in YS. The *GG* genotype was more common among the foreign breeds (YS, LD, and DR; frequencies of 0.8281, 0.6094, and 0.7106, respectively).

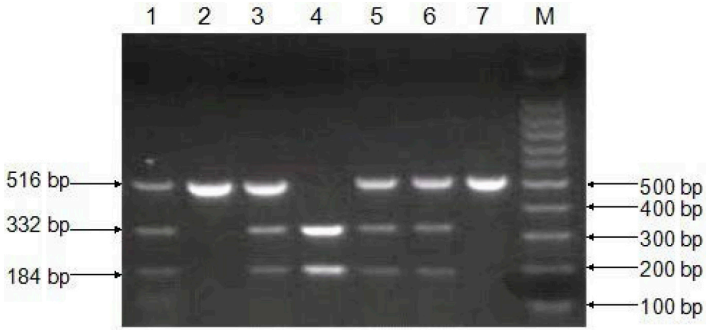

Figure 3. Agarose gel (2%) showing the genotypes of the only growth arrest-specific 6 (*GAS6*) gene single nucleotide polymorphism (SNP) identified in the pig sequence (516 bp for allele A, and 332 and 184 bp for allele G). Polymerase chain reaction (PCR) products were digested with *Bgl*I restriction enzyme to distinguish different alleles. *Lanes 1* to *7:.AG*, *AA*, *AG*, *GG*, *AG*, *AG*, and *AA; lane M*: DL100-bp DNA ladder.

Table 3. Frequency of alleles and genotypes of the growth arrest-specific 6 (*GAS6*) gene among different pig breeds

Breed	Sample size	Frequency of genotype			Frequency of allele	
		AA	*AG*	*GG*	*A*	*G*
NX	68	0.0148	0.3676	0.6176	0.1985	0.8015
DWZ	49	0.1020	0.6531	0.2449	0.4286	0.5714
SZL	77	0.0520	0.1688	0.7792	0.1364	0.8636
TY	69	0.0725	0.3478	0.5797	0.2464	0.7536
WZS	55	0.3091	0.4727	0.2182	0.5455	0.4545
YS	582	0.0723	0.0996	0.8281	0.1221	0.8779
LD	64	0.2187	0.1719	0.6094	0.3047	0.6953
DR	76	0.1447	0.1447	0.7106	0.2171	0.7829

Duroc (DR), Landrace (LD), Yorkshire (YS), Shaziling (SZL), Daweizi (DWZ), Taoyuan (TY), Ningxiang (NX), and Wuzhishan (WZS).

Expression profile analysis

To analyze the expression patterns of *GAS6* during the porcine weaning period, mRNA synthesized from ten tissues in different piglet breeds was analyzed using quantitative PCR. As shown in Figure 4, expression of the *GAS6* gene was abundant in the longissimus dorsi, crureus, and pancreas, modest in the lungs and kidneys, and minor in the heart, spleen, cecum, intestine, and liver. The GAS6 protein was detected in the longissimus dorsi tissue of SZL and YS piglets using immunohistochemistry, with an abundant protein expression index (PEI) (Figure 5). The GAS6 protein was expressed on the surface membranes of cells, illustrating that the GAS6 protein is a membrane protein and may be involved in signal transduction, cell adhesion, etc., in the weaned period. However, the results of GAS6 protein expression between the two pig breeds showed no statistical difference ($P > 0.05$). It has been reported that *GAS6* is significantly expressed in skeletal muscle (Hafizi and Dahlbäck, 2006), and feeding a high-fat fodder to a *GAS6*$^{-/-}$ mouse for a long time (12-15 weeks) resulted in a significantly lower adipose deposition rate compared to the control group; thus, *GAS6* can promote fat deposition and is associated with an increased number of adipose cells (Maquoi et al., 2005). The results from a previous study suggest that the mouse *GAS6*/*AXL* signaling pathway is related to the maintenance of energy metabolism and homeostasis after activation, with the inactivated pathway raising blood glucose levels and increasing fat deposition (Gao et al., 2014). *GAS6* was identified as the key gene in the *GAS6*/*AXL* signaling pathway, indicating that *GAS6* expression was higher in tissues involved in growth and fat deposition, including the longissimus dorsi, crureus, and pancreas, directly affecting the lean ratio of pigs (Gao et al., 2014).

GAS6 mRNA expression levels differed between two different genetic background breeds of pigs (YS and SZL) and among four tissue types. For tissues, *GAS6* expression was significantly higher in the longissimus dorsi and crureus compared to the spleen and liver ($P < 0.01$), but there were no differences in expression among the remaining tissues ($P > 0.05$). For the two breeds, expression of *GAS6* in the longissimus dorsi muscle, crureus, and spleen was significantly higher in SZL than in YS ($P < 0.05$), and in particular, expression of *GAS6* in the pancreas of SZL was significantly higher than in YS ($P < 0.01$). Significant differences in expression of *GAS6* among these tissues could be an indication of different growth rates between the foreign and indigenous breeds during the early growth period. Significantly higher expression of *GAS6* in SZL suggests that muscle tissue grows faster than in YS in the early growth period. *GAS6* mRNA expression was

detected in all tissues tested, indicating that porcine *GAS6* may be involved in certain metabolic processes to promote growth during early development; however, this requires further study.

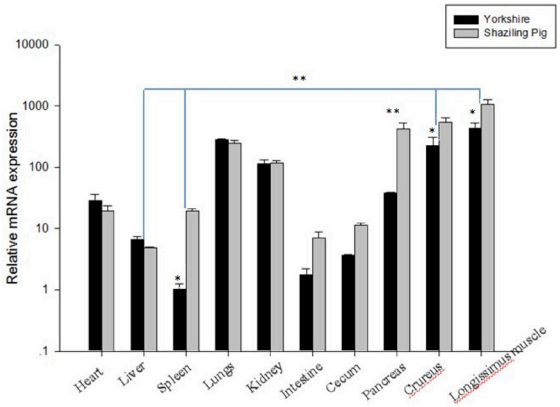

Figure 4. Comparison of *GAS6* mRNA expression profiles in different tissues from 25-day-old Yorkshire and Shaziling pigs. *P < 0.05, **P < 0.01.

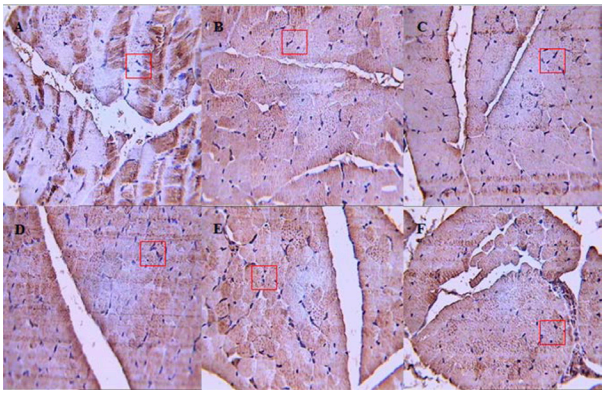

Figure 5. Expression of the GAS6 protein in longissimus dorsi tissue of pigs. **A** to **C**, Expression in Yorkshire piglets; **D** to **F**, expression in Shaziling piglets.

CONCLUSIONS

In summary, we obtained the complete cDNA sequence of the pig *GAS6* gene for the first time, and analyzed its predicted protein. One SNP in exon 12 of *GAS6* was identified, and the *G* allele was dominant in the pig breeds tested, with the *GG* genotype being more common among the foreign breeds. The expression profiles of pig *GAS6* mRNA displayed abundant expression in all tissues tested, especially in the longissimus dorsi, crureus, and pancreas, with different expression patterns observed between two weaned piglets with distinctive backgrounds. The immunohistochemistry results showed that the GAS6 protein was expressed abundantly in the longissimus dorsi tissue tested, suggesting that the *GAS6* gene may be involved in certain metabolic processes to promote growth during early development, and there is a high possibility that *GAS6* plays an important role in muscle. The lack of basic data on the *GAS6* gene and its

expression in porcine has impeded molecular studies. Our study provides novel findings on *GAS6* in porcine, which may be valuable to researchers of economical livestock. The results imply that the *GAS6* gene could be considered as a potential candidate gene for fat deposition in pigs.

Conflicts of interest

The authors declare no conflict of interest.

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