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ORIGINAL ARTICLE

Single Nucleotide Polymorphism Scanning and Expression of the Pig *PPARGC1A* Gene in Different Breeds

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Abstract Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PPARGC1A*) is a candidate gene for lean meat production because it plays a key role in lipid metabolism. In this study, SNPs within the porcine *PPARGC1A* gene were investigated using PCR-sequencing and PCR-RFLP. Quantitative real-time PCR and Western blot were then used to analyze mRNA and protein expression in *longissimus dorsi* muscle (LM), liver, and backfat tissues of Dianna small-ear pigs (DSP, $n = 6$), Tibetan pigs (TP, $n = 6$), and large white pigs (LW, $n = 6$). Five novel SNPs (*g.−1269A>G* in the 5′-upstream regulatory region; *g.190C>T*, *g.218C>A* and *g.234C>A* in exon 8; and *g.20C>T* in intron 10) and three previously identified SNPs (*g.417A>T* in exon 8; *g.56C>A* in exon 9; and *g.34G>A* in intron 9) were found. Of these, only two, *g.−1269A>G* and *g.234C>A*, had three different genotypes in the three breeds (DSP, $n = 63$; TP, $n = 51$; and LW, $n = 52$). Expression was highest in LM, modest in the liver, and minimal in backfat. In LM tissue, LW had higher mRNA and protein levels than DSP and TP ($P < 0.05$), and there was a negative correlation between gene expression and intramuscular fat (IMF) content. LW had numerically higher expression in liver and backfat tissues than DSP and TP, and the differences in protein levels were significant ($P < 0.05$ in liver, $P < 0.01$ in backfat). In conclusion, *PPARGC1A* may play a key role in down-regulating lipid deposition, and the SNPs with differential genotype distribution among the three pig breeds may be related to gene expression and fat deposition.

Keywords Pig · Lipids · *PPARGC1A* · Gene expression · SNPs

Abbreviations

IMF	Intramuscular fat
SNP	Single nucleotide polymorphisms
PCR-RFLP	PCR-restriction fragment length polymorphism
LM	*Longissimus dorsi* muscle
DSP	Dianna small-ear pig
LW	Large white pig
TP	Tibetan pigs
QTL	Quantitative trait loci
eECL	Enhanced electrochemiluminescence

Introduction

Fatness traits, such as backfat thickness and Intramuscular fat (IMF) content that influence meat quality and carcass composition, play an economic importance in the pork industry with influencing meat quality and carcass composition [1–3]. In past decades, pig breeding focused on

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selection of traits related to increasing the percentage of lean tissue, feed conversion, and growth rate. Nowadays, meat quality is increasingly valued in pig breeding because of customer preference. However, it is difficult to select pigs for improved meat quality while maintaining a lean carcass [4, 5] because selection for lean meat growth efficiency has adverse effects on meat quality [6, 7]. Identification of major genes affecting these qualities, followed by marker-assisted selection, is a feasible approach to improve meat quality in pig breeding.

Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A) is a transcriptional coactivator that controls the expression of a wide array of genes involved in glucose and fatty acid metabolism [8–10]. Expression of this gene is abundant in tissues that demand much energy, such as muscle, brown fat, and brain [11–13], as well as tissues that contribute to adipogenesis and adipocyte differentiation [14]. The porcine *PPARGC1A* gene (GenBank NO.NW_003540975.1 and NM_213963.1) is located on p2.1–p2.3 of chromosome 8 and contains 13 exons with a 90 bp 5′-UTR. The size of the coding region is 2,388 bp, encoding 796 amino acids. Several single nucleotide polymorphisms (SNPs) in the coding region and the 5′ and 3′ regulatory regions of porcine *PPARGC1A* gene have been identified and have been found to be associated with fat characteristics [15–20]. The mRNA expression of porcine *PPARGC1A* in several tissues and different ages of pigs has been described in previous reports [17, 18, 20, 21]. However, the protein expression pattern of *PPARGC1A* has not yet been reported.

The Diannan small-ear pig (DSP), a Chinese native breed from southern Yunnan province, has several special characteristics, such as high IMF content and tender meat. The Tibetan pig (TP) is a unique pig breed native to the Tibetan Plateau and mainly distributed in Linzhi and Changdu (Tibet), Hezuo (Gansu), Diqing (Yunnan), and Aba and Ganzi (Sichuan). In addition to being well-adapted to the harsh conditions in these areas, this pig also has a high IMF content [22, 23]. The Large White (LW), an introduced pig breed, possesses good traits in the areas of growth rate and lean ratio, but has a low IMF content [24, 25]. We proposed a hypothesis that the varying phenotype of fat traits in pig, such as backfat thickness and IMF content are related to different levels of *PPARGC1A* expression (mRNA or protein) or polymorphism of SNPs in the *PPARGC1A* gene region. The aim of this study was to survey the mRNA and protein expression patterns of *PPARGC1A* and to investigate genomic polymorphisms in the three pig breeds described above.

Materials and Methods

All experimental procedures were performed according to the Guide for Animal Care and Use of Laboratory Animals in the Institutional Animal Care and Use Committee of China Agricultural University. The experimental protocol was approved by the Department Animal Ethics Committee of China Agricultural University.

Animals and Samples

DSP, TP, and LW used in this study were raised under the same conditions. Six individuals in each group were slaughtered at 6 months of age, and the average body weight of DSP, TP and LW was 74.50 ± 1.05 kg (mean ± standard error), 30.53 ± 0.98 kg (mean ± standard error) and 105.32 ± 1.89 kg (mean ± standard error), respectively. Liver, *longissimus dorsi* muscle (LM) at the 12th rib, and backfat at the 12th rib were collected and immediately placed in RNAlater (Bioteke Co., Ltd., Beijing, China) or snap frozen in liquid nitrogen for extraction of total RNA and protein, and samples of LM (about 100 g) were collected for measurement of IMF content. Ear tissue samples of DSP ($n = 63$), TP ($n = 51$), and LW ($n = 52$) were collected and stored in 75 % alcohol for extraction of DNA.

Determination of IMF Content

The IMF content of the LM samples was determined after extraction of crude fat by Soxhlet extraction (SZF-06A, Shanhai xinjia electron Co., Ltd., Shanghai, China) with petroleum ether (boiling temperature range is from 60 to 90 °C). The Soxhlet extraction method, first established in 1879 by Franz von Soxhlet [26], provides highly accurate measurements with considerably shorter extraction time compared with other methods, as described by Tyra [27]. Two replications were done for every sample.

DNA, RNA, and Protein Extraction

Genomic DNA was isolated from ear tissues according to the extraction procedure described by Sambrook et al. [28] and preserved at −20 °C.

Total RNA was isolated from the liver, LM, and backfat tissues using the RNApure Tissue Kit (CWBIO Co., Ltd., Beijing, China). The RNA solutions were checked for concentration and purity using a NanoDrop 2000 biophotometer (Thermo scientific, USA), utilizing a 260/280 nm absorbance ratio (range 1.8–2.0 indicates a pure RNA sample). The integrity was then verified in a 1 % agarose gel. cDNA was synthesized from 2 μg total RNA using a SuperRT cDNA Kit (CWBIO Co., Ltd., Beijing, China) and was then stored at −20 °C.

Total protein was extracted using splitting solution (Urea 7 M, sul-Urea 4 M, DTT 60 mM, PMSF 10 mM). Protein concentration was assayed using the Super-Bradford Protein Assay Kit (CWBIO Co., Ltd., Beijing, China).

Table 1 Primers used for SNP identification and real-time PCR of the pig *PPARGC1A* gene

Primer name	Target region	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)	Amplicon length (bp)
5′-FR1	−1,459 bp/10 bp	AAGGGATGGATAGTAGGGA	AGCCCCTTACTGAGAGTGAAC	1,470
PGC-1	34 bp/intron 1 115 bp	GCTCTGTGTCACTGTGGATTG	ACTTTGCTGCCTCCTCTCC	222
PGC-2	Exon 2 and partial intron 2	TGCTGCTCTGGTTGGTGAAG	TATTAGTACGACCCAAGCCA	248
PGC-3	Partial exon 3 and intron 3	TGGAGATGTGACCACTGAGAA	TGTGCTTGGGGATTGTTTTG	217
PGC-4	Exon 4 and partial introns	GCTTTTCCTCTTTCTTGATG	AAACCCTTGCTACTTCTCCT	207
PGC-5	Exon 5 and partial introns	GCCTTGTTTTTAAGACCGAG	CCTTGTAAATGTTGCGACTG	220
PGC-6	Exon 6, 7 and partial introns	GAAAACAAATCCGAGAAACC	CTCATCCCCACAAAAAGTCC	331
PGC-7	Exon 8 and partial introns	GGCTTCGGGCACAGAGTCA	CTCATAGTTCTGGCAGCACC	1,203
PGC-8	Exon 9, 10 and partial introns	TGGTAATCTGGGGTTCACGG	GAAACCCTCCTGTAAAACGA	467
PGC-9	Partial exon 11 and 12	CAGACCTGACACAACACGGAC	CGTCCACAAAAGTACAGCTCG	537
PGC-10	Exon 13, partial intron 12 and 3′-UTR	AGGATACCTCTTACCAGGCTTG	CGTGTGTCTTCATGGAACTGCT	583
PGC-11	CDs region from 1,929 bp to 2,039 bp	GAAGAGGGAAGAATACCGC	CCAACATAAATCACACGACG	111
GAPDH	AF017079.1	GGTCACCAGGGCTGCTTTTA	CCTTGACTGTGCCGTGGAAT	134

SNP Screening

SNPs of the pig *PPARGC1A* gene were screened from the 5′ flanking region (−1,459 bp upstream of the transcription start site) to the 3′-UTR, including the coding region. The primers used for SNP screening are shown in Table 1. The amplicons generated using these primers covered the 5′ flanking region, all 13 exons, and partial intron regions. The PCR products amplified from ten pigs of each group were pooled and directly sequenced to identify SNPs. The sequencing chromatogram and assembly were analyzed by ChromasPro Version 1.33. Polymorphism of each SNP was detected using PCR-RFLP or examination of the PCR-amplified sequence.

Real-time PCR Assay with SYBR Green

Real-time PCR was conducted using the Bio-Rad CFX96 System (Bio-Rad, USA), with each reaction consisting of 10.0 μl 2 × SYBR Green qPCR SuperMix (Transgen, Beijing, China), 1.0 μl cDNA, and 0.3 μl of each primer (10.0 nmol/μl), supplemented with water to a total volume of 20 μl. The real-time PCR program started with a 20 s denaturation step at 95 °C, followed by 40 cycles of 5 s of denaturation at 95 °C and 15 s of annealing/elongation at 60 °C, during which fluorescence was measured. Next, a melting curve was constructed by increasing the temperature from 65 to 95 °C in sequential steps of 0.5 °C for 5 s, at which point fluorescence was measured. The real-time PCR efficiency of each primer pair was calculated with a 5 concentration-gradient of 5-fold dilution series of cDNA, which was used to construct a relative standard curve. PCR efficiencies ranged between 95 and 105 %. A cDNA pool from all samples was used as the normalization sample. The primers of PGC-11 (shown in Table 1) were used in real-time PCR for *Sus scrofa PPARGC1A* (GenBank NO. NM_213963.1). The primers for glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*; Table 1), used as a reference gene, were described in our previous report [29]. The gene expression quantity was calculated with the method of $2^{-\Delta\Delta C_t}$ [30].

Western Blot

The protein samples (40 mg each) were electrophoresed on 10 % SDS-PAGE gels and transferred to polyvinylidene difluoride membranes (Immobilon-FL membrane, 0.45 μm). Blots were blocked for 1.5 h at 37 °C in blocking buffer (TBS-T buffer containing 5 % blotting-grade nonfat dry milk), followed by incubation with the primary antibody of PPARGC1A (Biosynthesis biotechnology CO., Ltd., Beijing, China) or GAPDH (CWBIO Co., Ltd., Beijing, China) overnight at 4 °C and then with the horseradish peroxidase-labeled secondary antibody (goat anti-mouse IgG or goat anti-rabbit IgG) for 1.5 h at room temperature (RT). The membranes were washed for 5 min, three times, in TBS-T buffer (20 mmol/l Tris-HCl, 150 mmol/l NaCl, 0.5 % Tween-20). The membranes were drained briefly, then the enhanced electrochemiluminescence (eECL) reagents (CW0049A, CWBIO Ltd., China) were applied for 1 min (for GAPDH blots) or 5 min (for PPAGRC1A blots), then wrapped with cling film, put in an X-ray cassette, and exposed to X-ray film for 10 s (for GAPDH blots) or 10 min (for PPARGC1A blots). Blots were analyzed using ImageJ 1.44 software (NIH, USA) to determine expression ratio of PPARGC1A and GAPDH.

Table 2 IMF content of *longissimus dorsi* muscle in the three breeds

Pig breed	DSP	TP	LW
Number of samples	6	6	6
IMF content (%)	$2.98^{a} \pm 0.17$	$2.78^{a} \pm 0.10$	$1.10^{c} \pm 0.09$

Data are expressed as mean ± SE. The values in the same row with different superscript letters differ significantly ($P < 0.01$) and the same letter denotes no difference ($P > 0.05$)

Statistical Analysis

The Chi-square test was used to analyze the distribution balance of genotypes and the differences in allele frequencies. IMF contents and gene expressions were analyzed by one-way ANOVA, and a Bonferroni post hoc test assessed differences among groups using SAS9.1 Software (SAS Inst. Inc., Cary, NC). The results are presented as mean ± standard error. Significant and extreme differences were set at $P < 0.05$ and $P < 0.01$, respectively.

Results

IMF Content in Different Samples

IMF content ranges for the three pig breeds are listed in Table 2. DSPs had the highest IMF content, and LW had the lowest, of the three breeds. The IMF content of both DSP and TP was significantly higher than that of LW ($P < 0.01$).

SNP Detection and Polymorphism of SNPs

Eight SNPs were detected by PCR-sequencing: SNP1, g.−1269A>G (−1,269 bp upstream of the transcription start site); SNP2, g.190C>T Ala354Val (exon 8); SNP3, g.218C>A Gly363Gly (exon 8); SNP4, g. 234C>A Arg369Arg (exon 8); SNP5, g.417T>A Cys430Ser (exon 8); SNP6, g.56C>A Pro615Thr (exon 9); SNP7, g.34G>A (intron 9); and SNP8, g.20C>T (intron 10) (Table 3). The mutation, *g.−1269A>G* (−1,269 bp upstream of the transcription start site) was identified in the 5′ flanking region (Fig. 1a). To analyze polymorphism of this site (*g.−1269A>G*) in the sampled populations, a mismatched reverse primer with the sequence 5′-GTTCCA-GAGTGATGTATGCATGTACAAA-3′ was designed using dCAPS Finder 2.0 software (http://helix.wustl.edu/dcaps/dcaps.html). This mismatched reverse primer was used for PCR-RFLP analysis of the *g.−1269 A > G* site with the forward primer 5′-FR1, listed in Table 1. The PCR products were digested by the restriction enzyme *Ssp*I (AAT^ATT) into three fragments, 219, 190, and 29 bp, respectively (29 bp could not be detected by agarose gel, Fig. 1b).

The four SNPs located in exon 8 (g.190C>T Ala354Val; g.218C>A Gly363Gly; g.234C>A Arg369Arg; g.417T>A Cys430Ser) were detected by PCR-sequencing using the primer PGC-7 (shown in Table 1), and the other three SNPs (g.56C>A Pro615Thr exon 9; g.34G>A, intron 9 and g.20C>T, intron 10) were also detected using the primers PGC-8 (shown in Table 1). The frequencies of genotypes and alleles are shown in Table 3. All three genotypes of SNP1 and SNP4 could be detected in each of the three populations. There were no significant differences in allele distribution of SNP1 among the three breeds. However, for SNP4, the C allele frequency of LW was significant higher than that of DSP and TP ($P < 0.01$), and was higher in TP than in DSP ($P < 0.01$). The variant SNP2 was only detected in DSP and the variant SNP3 was only detected in TP. There were no variants of SNP5, SNP6, or SNP7 in DSP, and there were no variants of SNP8 in LW. The allele frequencies were not significantly different for SNP6 or SNP7 between TP and LW ($P > 0.05$). However, for SNP5, the A allele frequency was significant higher in TP than in LW ($P < 0.05$). For SNP8, the C allele frequency was much higher in TP than in DSP.

mRNA and Protein Expression of *PPARGC1A* in the Three Tissues

The *PPARGC1A* mRNA expression levels in the three tissues of LW were higher than those in DSP and TP (Fig. 2). In LM tissue, LW had significantly higher expression than both of the indigenous breeds (DSP and TP) ($P < 0.05$), while there were no significant differences between DSP and TP ($P > 0.05$). Comparing the mRNA expression levels of three tissues, the LM had maximal expression, the liver had modest expression, and the backfat had minimal expression. These differences were extreme ($P < 0.01$). The protein and mRNA expression levels of *PPARGC1A* were maximal in LW (Fig. 2).

Discussion

Genomics of the SNPs

Several quantitative trait loci (QTLs) for backfat thickness have been found near the region of the *PPARGC1A* gene [31, 32], indicating that this gene may be associated with lipid deposition. *PPARGC1A* could therefore be a candidate gene for lipid deposition and meat quality effects [33, 34]. In the present study, five novel SNPs (5′-flanking region, *A-1358G*; exon8, *C190T*, *C218A*, and *C234A*; and intron10, *C20T*) were found in the DSP, TP, and LW pig

Table 3 Genotype and allele frequency of the eight SNPs of *PPARGC1A* gene

SNP name	Mutation[a]	Breed	Genotype frequency (number/frequency)			Allele frequency		
			AA	AG	GG	χ^2 value[b]	A	G
SNP1	g.−1269A>G	DSP	46/0.73	12/0.19	5/0.08	7.247	0.75	0.25
		TP	26/0.51	21/0.41	4/0.08	0.0071	0.72	0.28
		LW	19/0.36	29/0.56	4/0.08	2.44	0.64	0.36
			TT	TC	CC	χ^2 value	T	C
SNP2	g.190C>T Ala354Val (exon8)	DSP	34/0.54	18/0.28	11/0.18	7.313	0.68	0.32
		TP	51/1	0/0	0/0	–	1	0
		LW	52/1	0/0	0/0	–	1	0
			CC	CA	AA	χ^2 value	C	A
SNP3	g.218C>A Gly363Gly (exon 8)	DSP	63/1	0/0	0/0	–	1	0
		TP	37/0.73	14/0.27	0/0	1.2908	0.86	0.14
		LW	52/1	0/0	0/0	–	1	0
			CC	CA	AA	χ^2 value	C	A
SNP4	g. 234C>A Arg369Arg (exon 8)	DSP	2/0.03	19/0.30	42/0.67	0.007	0.18	0.82
		TP	19/0.37	19/0.37	13/0.26	3.047	0.56	0.44
		LW	30/0.58	19/0.36	3/0.06	0.002	0.76	0.24
			AA	AT	TT	χ^2 value	A	T
SNP5	g.417 T>A Cys430Ser (exon 8)	DSP	63/1	0/0	0/0	–	1	0
		TP	44/0.86	6/0.12	1/0.02	1.768	0.92	0.08
		LW	25/0.48	20/0.38	7/0.14	0.826	0.67	0.33
			CC	CA	AA	χ^2 value	C	A
SNP6	g.56C>A Pro615Thr (exon 9)	DSP	63/1	0/0	0/0	–	1	0
		TP	42/0.82	2/0.04	7/0.14	36.999	0.84	0.16
		LW	45/0.86	5/0.10	2/0.04	7.983	0.91	0.09
			GG	GA	AA	χ^2 value	G	A
SNP7	g.34G>A (intron 9)	DSP	0/0	0/0	63/1	–	0	1
		TP	11/0.21	9/0.17	31/0.62	17.88	0.30	0.70
		LW	16/0.31	13/0.25	23/0.44	12.525	0.43	0.57
			CC	CT	TT	χ^2 value	C	T
SNP8	g.20C>T (intron 10)	DSP	3/0.05	2/0.03	58/0.92	33.854	0.06	0.94
		TP	36/0.71	1/0.02	14/0.27	46.204	0.72	0.28
		LW	52/1	0/0	0/0	–	1	0

[a] Loci of the SNPs were based on the first base of the exon or intron, and A-1269G was located 1,269 bp upstream of the transcription start site

[b] SNP was in complete linkage equilibrium when the Chi-square value was less than 3.84 ($P > 0.05$)

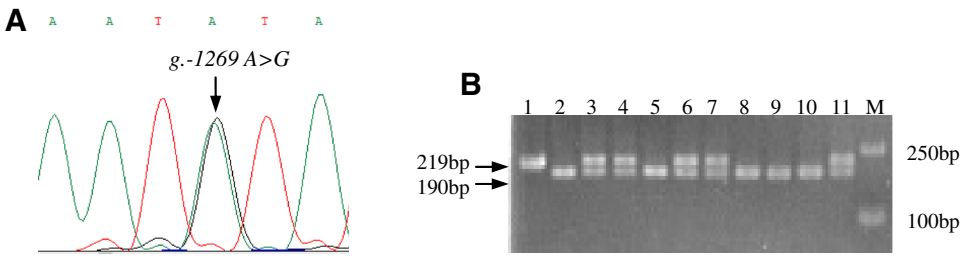

Fig. 1 Detection of SNP *g.−1269A>G* by sequencing and polymorphism analysis. **a** The sequencing chromatogram showing the site of SNP *g.−1269A>G*. **b** Agarose gel showing the amplified fragments containing SNP g.*−1269A>G,* digested with the *Ssp*I restriction enzyme. The *numbers on the gel* represent different genotypes. The *first lane* denotes GG genotype, *lanes 2, 5, 8, 9* and *10* denote AA genotype, and *lanes 3, 4, 6, 7* and *11* denote AG genotype. *Lane M* represents the DL2000 DNA marker

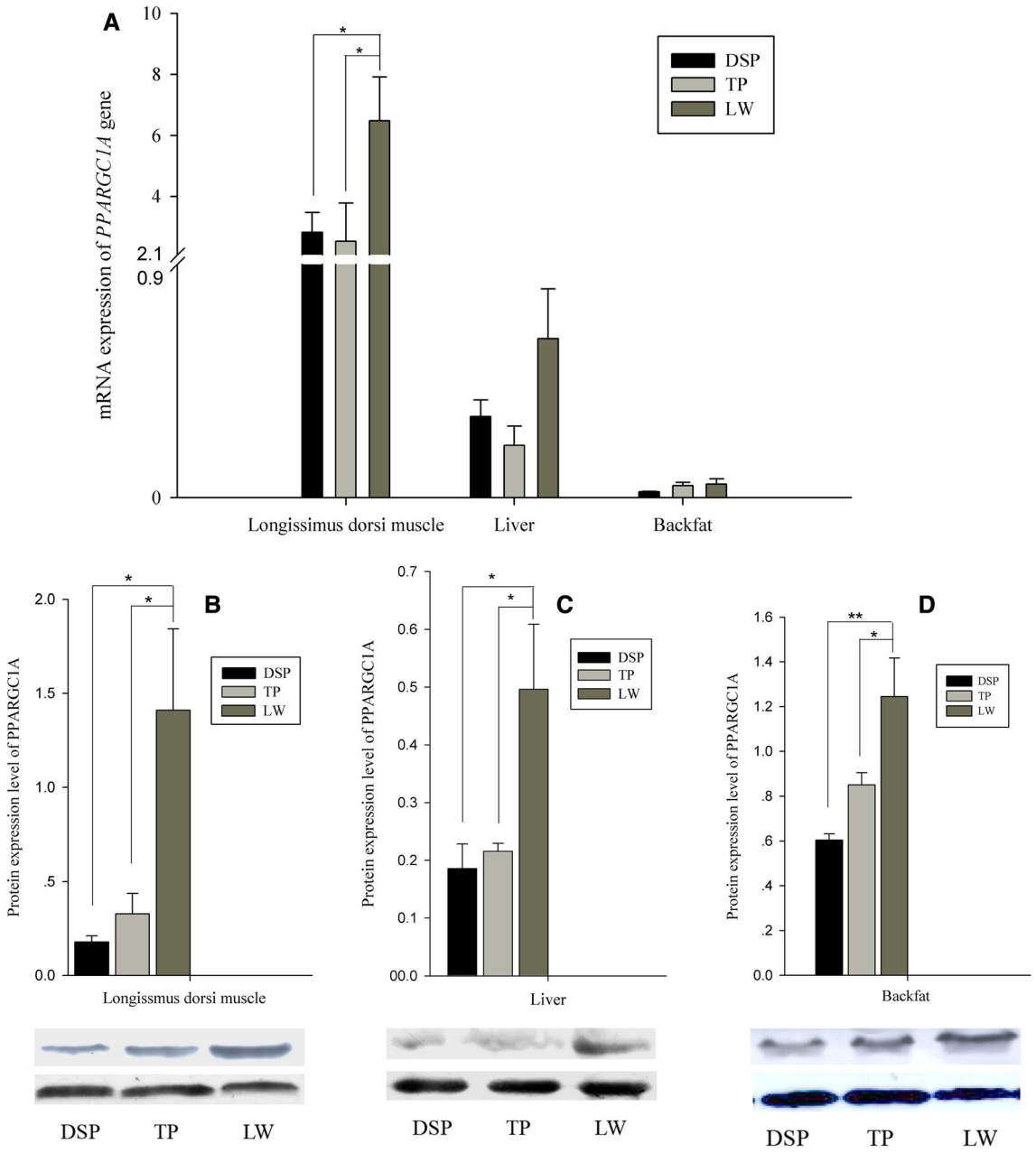

Fig. 2 The mRNA and protein expression levels of PPARGC1A in three tissues. *DSP* Diannan small-ear pig ($n = 6$), *TP* Tibetan pig ($n = 6$), and *LW* large white pig ($n = 6$). **a** The mRNA expression values were calculated using the ratio of test sample signal to normalization sample signal. Porcine *GAPDH* was used as the housekeeping gene. The break in the range on the *Y* axis in **a** represents the omission of 0.9–2.1. *Error bars* represent standard errors. *One and double stars* on bars denote significant differences in expression level ($P < 0.05$ and $P < 0.01$, respectively). **b–d** are the protein expression values were calculated using the ratio of gray level of *PPARGC1A–GAPDH* on the Western Blot membranes in LM, liver, and backfat tissues respectively

breeds. The transcription factor binding efficiency of the *g.−1269A>G* site was predicted using CONSITE (version 2.0) software, online (http://asp.ii.uib.no:8090/cgi-bin/CONSITE/consite) [35]. The results showed that the binding efficiency of two transcription factors, HFH-3 (Fork head homolog 3) and HFH-2 (Fork head homolog 2) which share homology in the winged helix DNA binding domain [36], were changed when the A mutated to G in the g.*−1269* site. HFH-3 binds the reverse sequence of the *PPARGC1A* gene when the site has the A base, but binding is disrupted when this base is mutated to G. HFH-2 binding efficiency was increased after mutation from A to G. We compared the amino acid sequences of PPARGC1A in bovin, human, rat and mouse species, which showed that

the Ala354 and Pro615 sites were conversed and the Cys430 site were different among these species. The *C190T* (Ala354Val) and *C218A* (Gly363Gly) polymorphisms in exon 8 were only found in DSP and TP, respectively. The *C234A* (Arg369Arg) polymorphism was detected in all three pig breeds, and the C allele frequency was significantly higher in LW than in DSP and TP. This SNP could be useful as a novel molecular marker for lipid deposition in subcutaneous fat and LM. The *A417T* (Cys430Ser) polymorphism was only found in LW and TP populations. However, Jacobs et al. (2006) [15] found no polymorphism at this site (*A417T*) in LW, Piétrain and Landrace populations (excluding the Meishan population). The *A417T* site has been significantly associated with the number and area of type I muscle fiber, as well as with muscle pH [37, 38], lightness [37], feed reward [16], and cooking loss [38]. Furthermore, seven SNPs in the 5′ upstream region [39] and two SNPs in the 3′-UTR [40] of the *PPARGC1A* gene were found to be associated with number and area of muscle fiber, respectively.

The *C56A* (Pro615Thr) polymorphism in exon 9 was associated with leaf fat weight, average backfat depth, and weight of the belly, and the *G34A* polymorphism in intron 9 was detected in Meishan, LW, Piétrain, and Landrace pigs [15]. In the present study, TP and LW had three genotypes for both of these SNPs, but DSP populations had only one genotype (CC genotype for C56A and AA genotype for G34A). The *C20T* polymorphism in intron 10 was only found in the Chinese native breeds (DSP and TP), and the T allele frequency (0.94) in DSP was significantly higher than that in TP (0.28). However, the T allele was not found in LW populations.

Expression of *PPARGC1A* mRNA and Protein

Although tissue expression of *PPARGC1A* has been studied [15, 17, 41, 42], little is known about comparative protein expression levels in different porcine tissues. In this study, the mRNA levels of *PPARGC1A* were highest in LM and lowest in backfat tissue, which is in accordance with the report from Erkens [17]. The mRNA and protein expression of *PPARGC1A* in LM was significantly higher in LW than in DSP and TP ($P < 0.05$). The same trend was seen in the protein expression patterns in liver and backfat tissues. The IMF content of DSP and TP was 2.7- and 2.5-fold higher, respectively, than that of LW. There was a negative correlation between expression of *PPARGC1A* and IMF content; the higher the *PPARGC1A* gene expression in LM, the lower the IMF content. The PPARGC1A was considered a positive regulator of pyruvate dehydrogenase kinase-4 (*PDK4*) [43–45], an inhibitor of pyruvate dehydrogenase [46, 47], that switched the energy supply from glucose to fatty acid oxidization [45]. This may be one of the reasons for the lower IMF content in LW vs. DSP and TP pigs, although the *PDK4* gene expression was not measured in this experiment. Liver that is a main place of lipid synthesis and delivery has important roles in lipometabolism, and fat tissues are places of lipid storage and mobilization. The *PPARGC1A* expressions in backfat and liver tissues were higher in LW than in DSP and TP, although the difference in mRNA abundance was not significant ($P > 0.05$), the protein level was significantly higher in LW than in DSP ($P < 0.05$ in liver; $P < 0.01$ in backfat) and TP ($P < 0.05$ in liver and backfat). This may be one of the reasons underlying the thinner backfat thickness in LW compared with DSP and TP. The gluconeogenesis mainly occurs in liver, which is induced by PPARGC1A with the forkhead transcription factor Foxo1 [48]. High levels of PPARGC1A in LW's liver tend to produce more glucose.

In conclusion, PPARGC1A may play a key role in lipolysis and fatty acid β-oxidization, and the SNPs with differential genotype distribution among the three pig breeds may affect gene expression and fat deposition. Further analyses of the correlation of these SNPs with gene expression and phenotypes of fatness and meat quality traits should be carried out to reveal the effects of these SNPs and to find meaningful molecular markers for improving meat quality.

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