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Association of *PGC-1α* Gene with Intramuscular Fat Content and Muscle Fiber Traits and Gene Expression in Tibetan Pigs

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Abstract: The *PGC-1α* gene was considered as one of the potential candidate genes for meat quality traits in pig considering its function of regulation in energy and fat metabolism. The aim of this study was to investigate the associations of the C430S polymorphic site of the *PGC-1α* gene with intramuscular fat content and muscle fibre area and perimeter in Tibetan pigs and the gene expression changes pattern at different ages. The C430S polymorphism was genotyped by PCR-RFLP using AluI restriction enzyme. Statistical analyses revealed that the C430S genotypes were significantly associated with intramuscular fat content ($p<0.01$) and significantly affected area and perimeter of muscle fibre ($p<0.05$, $p<0.05$). The gene expression levels of PGC-1α in heart, liver, spleen and kidney were examined in Tibetan pigs at 60, 90,120,150 and 180 days, respectively by quantitative real-time PCR. The result indicated that the mRNA abundance in heart and liver were increasing from 60-180 days ($p<0.01$, $p>0.05$) whereas in spleen and kidney, it were increasing from 60-120 days ($p<0.01$, $p<0.01$) then decreasing from 150-180 days ($p>0.05$, $p>0.05$).The mRNA abundance in heart and kidney were higher than in liver and spleen which was in agreement with the function of PGC-1α in adaptive thermogenesis. On the basis of these results, we suggested that the C430S polymorphism could be used as a meaningful molecular marker to improve meat quality.

Key words: *PGC-1α* gene, intramuscular fat, muscle fibre traits, gene expression, meat, quality, kidney, China

INTRODUCTION

Peroxisome Proliferative Activated Receptor Gamma Coactivator 1α (PPARGC1A), formerly known as PGC-1α was a cold and exercise inducible coactivator with a vital role in energy and fat metabolism. PGC-1α had a key role in linking nuclear receptors to the transcriptional program of adaptive thermogenesis, mitochondrial biogenesis and respiration, adipogenesis and adipocyte differentiation (Puigserver and Spiegelman, 2003), meanwhile, it was a principal factor regulating muscle fibre type determination (Lin *et al*., 2002). Therefore, *PGC-1α* gene maybe closely related with meat quality linking according to its function. Recently, there was a great interest in PGC-1α because rapidly growing evidences strongly suggested that it was a powerful candidate gene for pig meat quality. Different Single Nucleotide Polymorphisms (SNPs) in both coding and regulatory regions of PGC-1α have been independently associated with several fat characteristics in pigs (Jacobs *et al*., 2006; Stachowiak *et al*., 2007; Flisar *et al*., 2006; Erkens *et al*., 2009). There were also some studies found SNPs in PGC-1α associated with milk fat yield in cattle (Weikard *et al*., 2005; Kowalewska-Luczak *et al*., 2010) and with obesity/ type II diabetes in human (Ek *et al*., 2001; Vimaleswaran *et al*., 2006). Kunej *et al*. (2005) found an amino acid substitution (Cys to Ser) at position 430 of the amino acid sequence in exon 8 and found there were difference in the allelic frequencies on the locus between fat-type Chinese and lean-type Western pig breeds. Stachowiak *et al*. (2007) analysis C430S in 6 different pig breeds populations and found that the SNP revealed only related to the feed conversion ratio in the Polish Large White. Kim *et al*. (2010) revealed the C430S related with muscle fibre characteristics, meat pH and lightness in Yorkshire pigs.

Tibetan pig is a valuable and rare breed living on plateau. Because of long-term living on cold mountains made the meat quality of Tibetan pig had its special characteristics such as small muscle fibre and tender meat. Tibetan pigs mainly live on Qingzang plateau including Diqing (Yunnan province of China), Aba and Ganzi (Sichuan province of China), Linzhi and Changdu (Tibet Autonomous region of China).

The effects of the C430S genotypes on meat quality traits of the *PGC-1α* gene in fat-type Chinese pig breeds have not yet been examined. Therefore, the objectives of the present study were to reveal relationships between C430S and meat quality traits of the unique pig breed, Tibetan pig and to reveal the gene expression changes during postnatal growth.

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MATERIALS AND METHODS

Animals and samples: Animals were raised with *ad libitum* feeding and free drink water. The 72 unrelated Tibetan hogs were slaughtered at 60±1 kg living body weight and the longissimus dorsi muscles at the last thoracic vertebrae were dissected for analysis of muscle fibre area and perimeter and testing of Intramuscular Fat (IMF). The IMF were determined according to ISO 1444:1996 and the muscle fibre area and perimeter were measured as following: longissimus dorsi muscles were cut into 0.5×0.5×1.0 cm pieces, snap frozen in isopentane cooled by liquid nitrogen and stored at 80°C over night. Transverse serial sections (7 μm thick) were prepared in a cryostat (KD-VI; KEDEE) at -25°C and stained for Hematoxylineosin (HE). All muscle fibre samples were examined using an optical microscope equipped with a colour camera and a imagine analysis system (Motic images, Adwanced 3.2). Muscle fiber area and perimeter were calculated by Adwanced 3.2 automatically.

For analysis, the gene expression changes with ageing, 8 pigs were slaughtered at each postnatal stage: 60, 90, 120, 150 and 180 days, tissues samples such as heart, liver, spleen and kidney were collected and stored in liquid nitrogen for subsequent analysis of gene expression in different tissues of PGC-1α.

DNA analysis: TIANamp Genomic DNA Kit (Tiangen biotech Co., Ltd.) was used for genomic DNA isolation according to the manufacturer's protocol. The C430S polymorphism was genotyped by a PCR-RFLP method of Kunej *et al.* (2005).

Real-time PCR: Tissue samples were crushed to powder with liquid nitrogen and RNAprep pure tissue kit (Tiangen biotech Co., Ltd.) was used to isolate total RNA in the provided Elution solution according to the manufacturer's protocol. This kit also included an on-column DNase I treatment. The RNA quality was checked on agarose gel after which the RNA concentration (30-80 ng μL^{-1} and purity (OD260/280 ratio 1.8-2.1) were measured on a ND-2000 spectrophotometer (NanoDrop). For the RT reaction, approximately 1 μg of total RNA was converted into cDNA using the high-capacity cDNA reverse transcription kit (Applied-biosystems) according to the instructions manual. The cDNA synthesis was verified by a control PCR performed under the same conditions as the minus RT reaction. All PCR included a positive genomic and a negative no-template control.

Real-time PCRs were performed on MJ opticon2 (Bio-rad) with the QuantiTect SYBR green PCR kits (Qiagen). A melting curve was constructed to verify that only one gene-specific peak was present and to make sure that primer dimers were completely absent. The real-time PCR efficiency of each run was calculated with a 5 fold dilution series of cDNA which was used to construct a relative standard curve. Every run also included a no-template control. The qPCR mRNA expression measurement of each sample was performed in 3 duplicates. Primers for PGC-1α of real-time RT-PCR were as following: 5'-CCTGCATGAGT GTGTGCTCT-3', 5'-CTCAGAGTCCTGGTTGCA CA-3' (Jacobs *et al.*, 2006), the reference gene for normalization was ACTB and the primers were: 5'-TCTGGCACCACACCTTCT-3', 5'-TGATCTGGGTCATCTT CTCAC-3' (Erkens *et al.*, 2006).

Data analysis: The general linear model procedure was used to determine the associations between genotypes and traits, using the statistical software package SAS 9.13 (SAS Institute Inc.). The model was:

$$y_{ij} = \mu + g_i + e_{ij}$$

Where:

y_{ij} = Represented the observation
μ = The general mean
g_i = The fixed effect of genotype
e_{ij} = The random error

When significant differences were detected, the mean values were separated by the Probability Difference (PDIFF) option. The results were presented as least-square means together with standard errors. The $2^{-\Delta\Delta C}{}_T$ method was used for quantitative PCR data analysis (Livak and Schmittgen, 2001).

RESULTS AND DISCUSSION

Genotype and allele frequency of the C430S site: The frequencies of genotypes and alleles are shown in Table 1.

The TT genotyped animals had the highest frequency among genotypes and the frequency of allele T was much higher than A. This result was consistent with the founding of Kunej *et al.* (2005) which said that the mean frequency of allele T of Chinese pigs was ascendant whereas the frequency for two alleles of Western pigs were similar to each other.

Table 1: Genotype and allele frequencies for the C430S polymorphism of the *PGC-1α* gene in Tibetan pigs

	Genotype frequency			Allele frequency	
N	AA	AT	TT	A	T
72	0.111 (8)	0.25 (18)	0.639 (46)	0.236	0.764

Effect of C430S polymorphism on IMF, muscle fibre area and perimeter: The results of association analyses were shown in Table 2, the genotypes very significantly affected IMF of Tibetan pigs ($p<0.01$) which were closely linked to the functions of PGC-1α in regulating fat metabolism. TT genotypes had the highest and AA had lowest IFM.

Moreover, the association analyses also revealed that muscle fibre area and perimeter were significantly associated with the genotypes ($p<0.05$, $p<0.05$) which agreed with the results of Kim *et al.* (2010). This maybe connected with the function of *PGC-1α* gene for regulating muscle fibre type determination.

As we all know that the IMF and muscle fibre character both affect the taste of pork. Genotype TT had more IMF and more slender fibre which means that the C430S could be considered as a meaningful molecular marker to improve meat quality.

Gene expression pattern at different ages: The effect of ages on gene expression was also studied in this research (Fig. 1). During postnatal 60-180 days, the mRNA abundance in heart and liver were increasing. The gene expression level at 180th day was very significantly higher than that at 60-120th day in heart ($p<0.01$) but there was no significant difference at different ages in liver from 60-120 days, the gene expression level increased both in spleen and kidney ($p<0.01$, $p<0.01$) and from 150-180 days those deceased ($p>0.05$, $p>0.05$).

Gene expression pattern at different tissues: To determine whether porcine PGC-1α mRNA levels could be modulated according to tissues, samples were collected at heart, liver, spleen and kidney. Differences of expression levels were not significant at 60-90 days ($p>0.05$) between the four tissues whereas mRNA abundance in kidney was significantly higher than that in heart and liver ($p<0.05$) at 120th day and it was also extremely very significant higher than that in spleen at 150th day ($p<0.01$); the mRNA abundance in heart was very significant than that in spleen at 180th day ($p<0.01$). In totally, the gene expression level of PGC-1α in kidney at 120th day and in heart at 180 day got the highest ($p<0.05$, $p<0.01$) (Fig. 1). We detected PGC-1α transcription in heart, liver, spleen and kidney which quite agreed with the report of Jacobs *et al* . (2006).

Erkens *et al.* (2006) found there was a difference in porcine PGC-1α mRNA expression between different locations of longissimus dorsi muscle and backfat here in this study, we also found different expression pattern in four different tissues indicating that *PGC-1α* gene match its function maybe in the way of modulating transcription level. *PGC-1α* gene expression level in heart and kidney were higher than the other two tissues which is in agreement with the function of PPARGC1A in adaptive thermogenesis.

Table 2: Effects of C430S polymorphism on IMF (%) and muscle fibre area and perimeter in Tibetan pigs (LSM±SE)[1]

Genotypes	IMF (%)	Fibre area (μm²)	Fibre perimeter (μm)
AA	2.070±0.102[B]	2137.550±126.557[b]	252.970±11.921[b]
AT	2.816±0.110[B]	1654.077±57.822[a]	172.757±4.797[a]
TT	4.305±0.576[A]	1505.068±33.531[a]	165.823±3.231[a]

[1]Least squares means in the same column having different capital superscripts were significantly different ($p<0.01$) or having different lowercases were different ($p<0.05$)

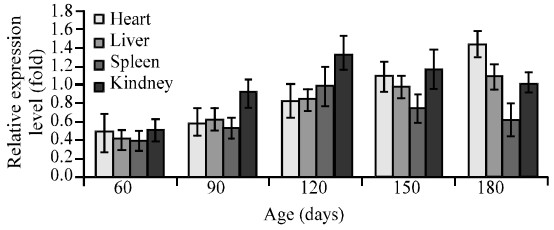

Fig. 1: Tibetan pig *PGC-1α* gene expression changes during postnatal growth

CONCLUSION

The current study showed that in Tibetan pigs, the C430S polymorphism of *PGC-1α* gene had very significant effect on IMF ($p<0.01$) and significantly associated with muscle fibre area and perimeter ($p<0.05$, $p<0.05$).

The detection of *PGC-1α* gene expression level revealed that the mRNA abundance in heart and kidney were higher than liver and spleen which was in agreement with the function of PGC-1α. Accordingly, we suggest that the polymorphism of C430S can be used as a meaningful molecular marker for improving meat quality.

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