Journal of Animal and Veterinary Advances 10 (5): 597-600, 2011
ISSN: 1680-5593

Expression Changes of PPARGC1A During the Development of Lean and Obese Pigs

An-An Jiang, Ming-Zhou Li, Kai Zhang, Yi-Ren Gu and Xue-Wei Li
Institute of Animal Genetics and Breeding,
College of Animal Science and Technology,
Sichuan Agricultural University, 625014, Ya'an, China

Abstract: PPARGC1A, the gene encoding peroxisome proliferative activated receptor-γ coactivator-1α is a useful candidate gene for pork quality due to its regulation of the determination of myofibre type. In this study, changes in developmental gene expression of PPARGC1A in longissimus dorsi muscles were examined in two pig breeds, Landrace (lean) and Meishan pigs (obese) at birth, 30, 60, 90, 120 and 150 days of age using quantitative real-time PCR. The results indicated that there were distinct expression patterns of PPARGC1A in the two pig breeds. At most stages, except at 30 and 60 days, the abundance of PPARGC1A mRNA in Meishan pigs was significantly higher than that in Landrace ($p<0.01$). Interestingly, there was a negative correlation between the PPARGC1A expression pattern and myofibre cross-sectional area across five developmental stages which implies that PPARGC1A might have an important influence on myofibre growth.

Key words: Myofibre cross-sectional area, pig, PPARGC1A, q-PCR, expression, China

INTRODUCTION

Peroxisome proliferative activated receptor-γ coactivator-1α (PPARGC1A), a nuclear transcriptional factor plays an important role in hepatic gluconeogenesis (Yoon *et al*., 2001), mitochondrial biogenesis and respiration (Puigserver *et al*., 1998), adipogenesis and adipocyte differentiation (Spiegelman *et al*., 2000) and metabolic homeostasis (Puigserver, 2005). PPARGC1A interacts with other nuclear hormone receptors such as the glucocorticoid receptor (Knutti *et al*., 2000) oestrogen receptor (Tcherepanova *et al*., 2000), retinoic X receptor (Delerive *et al*., 2002) and glucose transport protein 4 (Michael *et al*., 2001). The abundance of PPARGC1A is closely associated with the number of oxidative myofibres making it a remarkable candidate gene for pork quality (Wu *et al*., 1999; Lin *et al*., 2002; Erkens *et al*., 2006).

Western pig breeds have been intensively selected over the past two decades for rapid and efficient accretion of muscle which is believed to have led to deterioration in meat quality as the extra muscle bulk contains fewer oxidative fibres and more glycolytic fibres. Landrace, a typical lean-type western breed is now widely used for commercial production throughout the world. While indigenous Chinese pig breeds have lower growth rates and lower lean meat content than conventional western pig breeds, they have proved superior in terms of perceived meat quality. The Meishan variety is a typical indigenous Chinese breed of pig. In general, the Intramuscular Fat content (IMF) of Meishan was higher than in the Landrace, meanwhile the shear force, muscle fibre diameter were lower than in the Landrace (Liu *et al*., 2006). This was the reason that resulted in better meat quality of Meishan pigs than Landrace.

Myofibres were considered as an important factors influencing meat quality and can be characterized by their total number, myofibre Cross-Sectional Area (CSA), length and contractile and metabolic types (Lefaucheur, 2006).

Researcher reported that increasing CSA is detrimental for meat quality especially in water holding capacity and tenderness (Rehfeldt *et al*., 2000). In this study, we surveyed the developmental gene expression patterns of PPARGC1A and changes in tendencies of CSA for the longissimus dorsi muscle in Landrace and Meishan pigs at birth, 30, 60, 90, 120 and 150 days.

MATERIALS AND METHODS

Total 48 pigs (24 Landrace and 24 Meishan pigs) were used in this study. The piglets were raised with *ad libitum* access to feed under normal conditions and were simultaneously weaned at 28±1 days of age. The animals were reared in compliance with national regulations for the humane care and use of animals in research. The pigs

Corresponding Author: Xue-Wei Li, Institute of Animal Genetics and Breeding, College of Animal Science and Technology, Sichuan Agricultural University, Ya'an 625014, China

Table 1: Primers used for quantitative real-time PCR

Gene symbol	Primer sequence	Product size	GenBank No.
PPARGC1A	F-5' CCTGCATGAGTGTGTGCTCT3' R-5' CTCAGAGTCCTGGTTGCACA3'	107 bp	AB106108
ACTB*	F-5' TCTGGCACCACACCTTCT 3' R-5' TGATCTGGGTCATCTTCTCAC 3'	114 bp	DQ178122
TBP*	F-5' GATGGACGTTCGGTTTAGG 3' R-5' AGCAGCACAGTACGAGCAA 3'	124 bp	DQ178129
TOP2B*	F-5' AACTGGATGATGCTAATGATGCT 3 R-5' TGGAAAAACTCCGTATCTGTCTC 3'	137 bp	AF222921

*:ACTB, TBP and TOP2B are the internal control genes

were sacrificed at a commercial slaughterhouse at six postnatal stages: birth, 30, 60, 90, 120 and 150 days of age. At each time point we sampled four pigs (two sows and two boars) for each breed. The longissimus dorsi muscle near the last 3rd or 4th rib was rapidly and manually dissected from each cleaved pig. A sample of the muscle was reserved for immediate microscopy and the rest was crushed to a powder in liquid nitrogen, subdivided into 80-100 mg portions and stored at -70°C until further use.

The muscles samples were fixed in 10% neutral buffered formalin solution, embedded in paraffin using TP1020 semi-enclosed tissue processor (Leica), sliced at a thickness of 6 μm using RM2135 rotary microtome (Leica) and stained with Haematoxylin and Eosin (H and E). The myofibre Cross-Sectional Area (CSA) was counted for an average of 100 fibres in randomly selected fields using a TE2000 fluorescence microscope (Nikon) and Image Pro-Plus 6.0 software (Media-Cybernetics).

Total RNA was extracted from -70°C stored samples of longissimus dorsi muscle with RNeasy Fibrous Tissue Min kit (Qiagen) according to the manufacturer's protocol. The purified RNA concentration was quantified using a photometer (Bio-Rad); the ratio of optical densities at 260 and 280 nm was between 1.8 and 2.0. RNA integrity was verified by loading RNA onto a 1% agarose gel. Subsequently, the RNA samples were prepared as follows: for each time point and each breed, equal quantities (10 μg) of total RNA isolated from four individual pigs were pooled. Approximately 40 μg of total RNA representing each postnatal stage and each breed was used for next quantitative real-time PCR (q-PCR) analysis.

The q-PCR analysis was performed on the PPARGC1A gene normalised to three internal control genes (beta-actin (ACTB), TATA Box Binding Protein (TBP) and topoisomerase II beta (TOP2B)) (Erkens *et al*., 2006). Primers for the four target sequences were designed using the Primer 3 online platform (http://frodo.wi.mit.edu/) (Table 1). Standard curves for above four genes were prepared from 10 fold dilution series of purified amplicons. The q-PCR reaction was conducted on an iQ5 real-time PCR detection system (Bio-Rad) using he SYBR PrimeScript RT-PCR kit (TaKaRa). All experiments contained a negative control and all q-PCR reactions were performed in triplicate. Normalisation Factors (NF) of three internal control genes were calculated using the geNorm tool (Vandesompele *et al*., 2002). The relative mRNA levels of PPARGC1A were normalised by dividing the raw expression data of the objective gene by the NF (Erkens *et al*., 2006). Statistical analyses were performed in SAS 8.0. The linear model of the response variable was as followed:

$$Y_{ijk} = breed_i + age_j + breed \times age_{(ij)} + e_{ijk}$$

Where:

Y_{ijk} = Relative mRNA levels
$breed_i$ = Fixed effects of breed
age_j = Fixed effects of age
$breed \times age_{(ij)}$ = Interaction effects of breed and age
e_{ijk} = Residual error

The differences in abundance of PPARGC1A expression at the interactions among breed and age were tested based on least squares means using t-test. The Pearson correlation coefficient (r) was calculated for each time point and each breed of PPARGC1A level and CSA with linear model.

RESULTS AND DISCUSSION

As shown in Fig. 1a, the CSA value increased constantly during all developmental stages in both pig breeds. The CSA of Landrace was higher than in the Meishan pigs at most stages except at 90 days. But at each time point, there were no significant differences between Landrace and Meishan pigs. This is consistent with the well-known fact that the meat quality of Meishan pigs is better than Landrace.

As shown in Fig. 1b, the changes in expression of PPARGC1A during developmental stages were distinct in the two pig breeds. In Landrace, PPARGC1A was expressed at a relatively low level at birth was up-regulated to a maximum at the age of 30 days and subsequently slightly down-regulated from 60-150 days. By comparison, in Meishan pigs, PPARGC1A expression

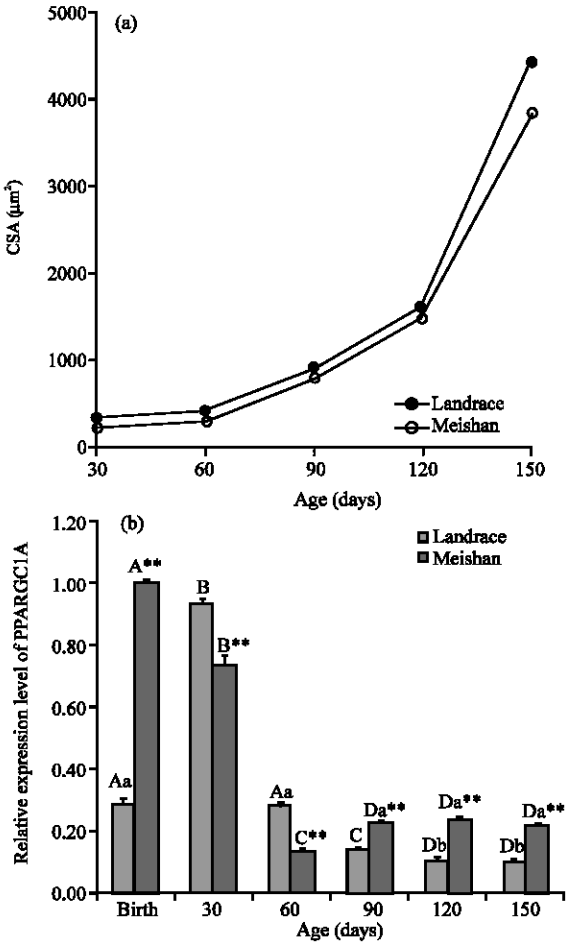

Fig. 1: (a) Developmental changes of CSA in two pig breeds; Developmental pattern of PPARGC1A mRNA in longissimus dorsi muscle for two pig breeds. The breed by age interaction is high significant ($p<0.01$). The same letter indicates no significant difference between six developmental stages within each pig breed; a different letter indicates significant difference at the 5% (small letter) or 1% (capital letter) levels. (b) **Indicates significant difference at the 1% level between Landrace and Meishan pigs at the same time point

was detected at its highest level at birth was down-regulated to a minimum at the age of 60 days and then remained at a steady level from 90-150 days.

There were significant differences in PPARGC1A mRNA abundance between the two pig breeds at all time points ($p<0.01$). We found that the abundance PPARGC1A mRNA was higher in Meishan pigs than that in Landrace at most stages (e.g., birth, 90, 120 and 150 days) which is consistent with previous reports that stated that higher levels of PPARGC1A mRNA were found in pig breeds possess better meat quality (Zhao *et al*., 2004). We also found that there was a negative correlation between PPARGC1A expression and CSA over five developmental stages (30-150 days) for Landrace ($r = -0.50$, Pearson) and Meishan pigs ($r = -0.35$, Pearson). A possible explanation for the results is that the pigs which expressed higher mRNA level of PPARGC1A characterized by lower CSA. Researchers reported that different Single Nucleotide Polymorphisms (SNPs) of PPARGC1A could be associated with porcine production traits.

The Pro615Thr polymorphism located in exon 9 of PPARGC1A could influence the meat quality (Erkens *et al*., 2010). It was reported the frequency distribution of a Cys430Ser (T/A substitution) polymorphism of PPARGC1A exhibit significant difference in Chinese and Western pig breeds but no relation with carcass composition was found in this study (Kunej *et al*., 2005; Stachowiak *et al*., 2007). In Landrace and Meishan pigs, there may be exist the different variants of PPARGC1A while its effect on the meat quality is unclear. Interestingly, the Meishan pigs possess the lower CSA and higher mRNA level of PPARGC1A than Landrace which indicated Meishan pigs have a better meat quality than Landrace. Nonetheless, PPARGC1A was considered to be principle factor regulating the determination of myofibre type (Lin *et al*., 2002; Zhao *et al*., 2004). Further efforts should focus on the mechanism by which PPARGC1A acts as an inhibitor of myofibre growth.

CONCLUSION

The study indicated that there were distinct expression patterns of PPARGC1A in two pig breeds that differ in meat quality and growth rate. There were higher PPARGC1A mRNA abundance and lower CSA in the Meishan pigs that possess a better meat quality. The results highlight that the PPARGC1A could be regarded as a candidate gene involved in affecting the CSA and meat quality. We envisage that this study will contribute to the elucidation of the relationship between pork quality and PPARGC1A expression.

ACKNOWLEDGEMENTS

This research was supported by grants from the National Special Foundation for Transgenic Species of China (2009ZX08009-155B; 2008ZX08006-003), the National Natural Science Foundation of China (30771534; 30901024) and the Specialized Research Fund of Chinese Ministry of Agriculture (NYCYTX-009).

REFERENCES

Delerive, P., Y. Wu, T.P. Burris, W.W. Chin and C.S. Suen, 2002. PGC-1 functions as a transcriptional coactivator for the retinoid X receptors. J. Biol. Chem., 277: 3913-3917.

Erkens, T., M. van Poucke, J. Vandesompele, K. Goossens, A. van Zeveren and L.J. Peelman, 2006. Development of a new set of reference genes for normalization of real-time RT-PCR data of porcine backfat and longissimus dorsi muscle and evaluation with PPARGC 1 A. BMC Biotechnol., 6: 41-41.

Erkens, T., S.D. Smet, K.V.D. Maagdenberg, A. Stinckens, N. Buys, A. van Zeveren and L.J. Peeman, 2010. Association analysis of PPARGC1A mutations with meat quality parameters in a commercial hybrid pig population. Czech J. Anim. Sci., 55: 200-208.

Knutti, D., A. Kaul and A. Kralli, 2000. A tissue-specific coactivator of steroid receptors, identified in a functional genetic screen. Mol. Cell Biol., 20: 2411-2422.

Kunej, T., X.L. Wu, T.M. Berlic, J.J. Michal, Z. Jiang and P. Dovc, 2005. Frequency distribution of a Cys430Ser polymorphism in peroxisome proliferator-activated receptor-gamma coactivator-1 (PPARGC1) gene sequence in Chinese and Western pig breeds. J. Anim. Breed. Genet., 122: 7-11.

Lefaucheur, L., 2006. Myofibre typing and its relationships to growth performance and meat quality. Archiv Tierzucht, 49: 4-17.

Lin, J., H. Wu, P.T. Tarr, C.Y. Zhang and Z. Wu *et al*., 2002. Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres. Nature, 418: 797-801.

Liu, S.D., M.Z. Liu, W.T. Wang, L. Han, X.P. Sun and G.S. Chen, 2006. Research of the histological characteristic of longissimu dorsi about several main breeds of swine in Ningxia. J. Agric. Sci., 27: 33-37.

Michael, L.F., Z. Wu, R.B. Cheatham, P. Puigserver and G. Adelmant *et al*., 2001. Restoration of insulin-sensitive glucose transporter (GLUT4) gene expression in muscle cells by the transcriptional coactivator PGC-1. Proc. Natl. Acad. Sci. USA., 98: 3820-3825.

Puigserver, P., 2005. Tissue-specific regulation of metabolic pathways through the transcriptional coactivator PGC1-α. Int. J. Obes., 1: 5-9.

Puigserver, P., Z. Wu, C.W. Park, R. Graves, M. Wright and B.M. Spiegelman, 1998. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell, 92: 829-839.

Rehfeldt, C., I. Fiedler, G. Dietl and K. Ender, 2000. Myogenesis and postnatal skeletal muscle cell growth as influenced by selection. Livestock Prod. Sci., 66: 177-188.

Spiegelman, B.M., P. Puigserver and Z. Wu, 2000. Regulation of adipogenesis and energy balance by PPARgamma and PGC-1. Int. J. Obes. Relat. Metab. Disord., 4: 8-10.

Stachowiak, M., M. Szydlowski, J. Cieslak and M. Switonski, 2007. SNPs in the porcine PPARGC1a gene: Interbreed differences and their phenotypic effects. Cell. Mol. Biol. Lett., 12: 231-239.

Tcherepanova, I., P. Puigserver, J.D. Norris, B.M. Spiegelman and D.P. McDonnell, 2000. Modulation of estrogen receptor-α transcriptional activity by the coactivator PGC-1. J. Biol. Chem., 275: 16302-16308.

Vandesompele J, K. De Preter, F. Pattyn, B. Poppe, N. van Roy, A. De Paepe and F. Speleman, 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol., 3: Research0034.1-Research0034.11.

Wu, Z., P. Puigserver, U. Andersson, C. Zhang and G. Adelmant *et al*., 1999. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell, 98: 115-124.

Yoon, J.C., P. Puigserver, G. Chen, J. Donovan and Z. Wu *et al*., 2001. Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. Nature, 413: 131-138.

Zhao, R.Q., X.J. Yang, Q.F. Xu, X.H. Wei, D. Xia and J. Chen, 2004. Expression of GHR and PGC-1 alpha in association with changes of MyHC isoform types in longissimus muscle of Erhualian and Large White pigs (Sus scrofa) during postnatal growth. Anim. Sci., 79: 203-211.