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Different Expression Patterns of Heart and Adipocyte Fatty Acid-Binding Protein Gene During Porcine Skeletal Muscle Development

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Abstract: The pig FABP3 (heart Fatty Acid-Binding Protein) and FABP4 (adipocyte Fatty Acid-Binding Protein) genes play an important role in intracellular fatty acid transport and considered to be candidate genes for Intramuscular Fat content (IMF) trait in pigs. In this study, the expression profiling of *FABP3* and *FABP4* genes was investigated in two pig breeds differing in muscularity (Yorkshire and Meishan) at four stages (fetal 65 days and postnatal 3, 60 and 120 days). The expression of FABP3 and FABP4 was significantly different in porcine skeletal muscle among different developmental stages and between the two breeds. This result suggests that the pig *FABP3* and *FABP4* may be important genes for meat quality and provides useful information for further studies on their roles in skeletal muscle intramuscular fat deposit.

Key words: Fatty acid binding protein, pig, skeletal muscle, breeds, muscularity, gene

INTRODUCTION

The proteins FABP3 (heart Fatty Acid Binding Protein) and FABP4 (adipocyte Fatty Acid Binding Protein) belongs to family of intracellular fatty acid binding proteins which play an important role in intracellular fatty acid transport (Chmurzynska, 2006). In pig, *FABP3* and *FABP4* genes have been considered as candidate genes for pig fatness traits (Gerbens *et al*., 2000; Urban *et al*., 2002). Mutant alleles of FABP3 and FABP4 have also been reported with a correlation with Intramuscular Fat (IMF) content in pigs (Lee *et al*., 2010; Li *et al*., 2010; Gerbens *et al*., 2001). Moreover, porcine *FABP3* and *FABP4* genes have been physically mapped on pig chromosme 6 (SSC6) (Oliver *et al*., 2002) where many significant Quantitative Trait Loci (QTL) affecting meat quality and carcass traits (backfat thickness and intramuscular fat content) have been identified.

Different breeds have different genetic background with different muscularity and pork quality. Western pig breed (Yorkshire) has been intensively selected over the past two decades for faster growth rate, higher feed efficiency and more lean meat content which is believed to have led to deterioration in meat quality. The indigenous Chinese pig breed (Meishan) is just the opposite but has proved superior in terms of perceived meat quality, especially in high Intramuscular Fat (IMF) content (White *et al*., 1995). So, comparative analysis of gene different expression patters in different breeds to understand the molecular mechanisms responsible for breed specific differences in meat quality is necessary and crucial. However, little is known the mRNA levels of *FABP3* and *FABP4* gene in Yorkshire and Meishan pigs during skeletal muscle development.

In this study, pig *FABP3* and *FABP4* gene expression differences were detected in muscle samples from different developmental stages in lean and fatty pig breed. The findings of transcriptional characterization of porcine *FABP3* and *FABP4* genes will undoubtedly help in further understanding theirs roles in porcine muscle intramuscular fat deposit.

MATERIALS AND METHODS

Animal and tissue: All animal procedures were performed according to protocols approved by Sichuan province, P.R. China for Biological Studies Animal Care and Use Committee. The longissimus dorsi muscle samples were collected from fetuses of Yorkshire and Chinese indigenous Meishan pregnant females at 65 days post conception (65 dpc) and three different postnatal periods (3, 60 and 120 days after birth, four samples at each stage) and then immediately frozen in liquid nitrogen and stored at -80°C.

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RNA preparation: Total RNAs were isolated from pig muscle tissues with Trizol reagent (Invitrogen) and treated with RNase-free DNase I (Takara) to remove contaminating genomic DNA. Nucleicacid concentrations were measured at 260 nm with a BioPhotometer (Eppendorf, Germany). Purity of the total RNA was determined by the A260/280 and A260/230 ratio and its integrity was tested by electrophoresis using 1% formaldehyde denaturing agarose gel.

cDNA synthesis and quality confirmation: The first strand cDNAs was synthesized using AMV reverse transcriptase (Promega, Madison, WI, USA) in a 50 μL reaction mixture according to the manufacturer's instruction. Briefly, a mixture of 2 μg total RNA, 5 μL oligo (dT) was incubated at 70°C for 5 min to break the RNA secondary structure. The mixture was then chilled on ice for at least 2 min and then 10 μL 5×RT buffer, 5 μL dNTPs (10 mM each), 50 U RNase Inhibitor and 200 U AMV reverse transcriptase were added for a total volume of 50 μL. The RT mix was incubated at 42°C for 60 min. Finally, the reverse transcriptase was inactivated by 5 min incubation at 90°C.

Quantitative real time RT-PCR analysis: Real-time RT-PCR was used to quantify the expression level of porcine FABP3 and FABP4 in four developmental stages using ABI 7300 real-time PCR thermal cycle instrument (ABI, USA) according to the supplied protocol. Each real-time PCR (in 25 μL) reaction contained 12.5 μL SYBR® Green Real time PCR Master Mixture (contains ROX Dye. Toyobo, Jap), 0.25 μM primers (FABP3-F, FABP3-R for FABP3 and FABP4-F, FABP4-R for FABP4, Table 1) and 1 μL normalized template cDNA. The cycling conditions consisted of an initial, single cycle for 3 min at 95°C followed by 40 cycles of cycling consisting of 20 sec at 94°C, 20 sec at 58°C, 15 sec at 72°C and final extension for 5 min. The specificity of PCR products were confirmed by melting curve analysis. Gene expression levels were quantified relatively to the expression of β-actin using Gene Expression Macro software (ABI, USA) by employing an Optimized Comparative Ct (2-ΔΔCt) Value Method (Livak and Schmittgen, 2001). All PCR amplifications were performed in triplicate for each RNA sample.

Table 1: Primer sequences used in this study

Gene name	Primer name	Primer sequence (5'-3')	Size (bp)	Tmp. (°C)
FABP3	FABP3-F	GAGTTTGATGAGACAACAGCAG	188	58
	FABP3-R	TCTTTCTCGTAAGTGCGAGTGC		
FABP4	FABP4-F	CTGAGATTGCCTTCAAATTG	223	58
	FABP4-R	TTGGCTTATGCTCTCTCATA		
β-actin	Actb-F	GGTCAAGCAGCATAATCCAAAG	158	60
	Actb-R	CAAGGGCATAGCCTACCACAA		

Statistical analysis: One-way ANOVA was employed using SPSS Version 13.0 to compare the difference of gene expression in different developmental stages and Duncan's new multiple rang test was used to analyze statistical significance. While Paired-Sample t-test was carried out to identify the expression differences at each stage between the two breeds $p<0.05$ was considered as significant.

RESULTS AND DISCUSSION

Differential expression of FABP3 during porcine skeletal muscle development between breeds: To analyze expression patterns of FABP3 during porcine skeletal muscle development, we performed the qRT-PCR analyses of four skeletal muscle developmental stages. As shown in Fig. 1, porcine FABP3 was expressed at 65 dpc with a very low level then increased to a peak at 3 days and was significantly down-regulated at 60 days ($p<0.05$) then increased at the 120 days after birth in Meishan pigs. The expression trend of FABP3 in the four skeletal muscle developmental stages in Yorkshire is generally coincident

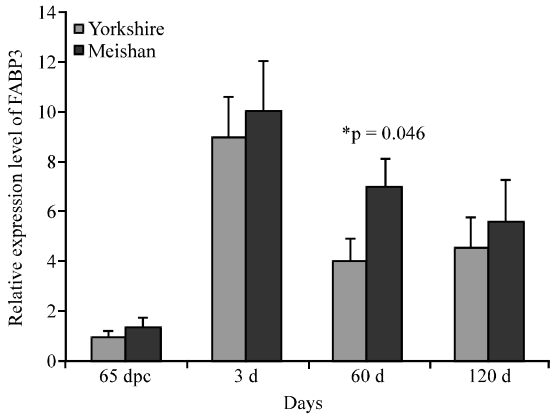

Fig. 1: The *FABP3* gene expression in the skeletal muscle from four different stages of Yorkshire and Meishan pigs by qRT-PCR. The expression level was normalized to β-actin and measured with 2^(-ΔΔCt) value. Results are averaged from three independent replicates during all stages. Error bars represent SD (n = 3). Significant levels were analyzed by t-test. ** = $p<0.01$; * = $p<0.05$. 65 dpc: embryonic day 65; 3 d: muscle from 3 days after birth; 60 d: muscle from 60 days after birth; 120 d: muscle from 120 days after birth

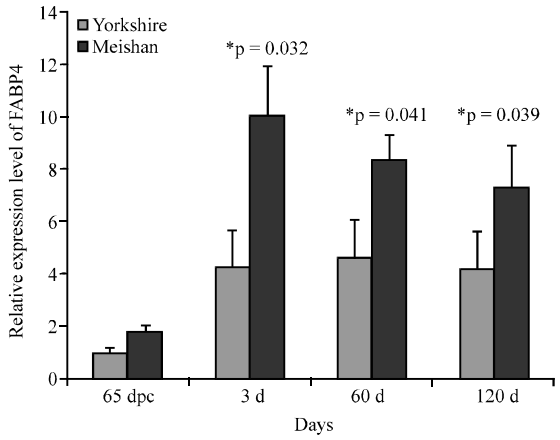

Fig. 2: The *FABP4* gene expression in the skeletal muscle from four different stages of Yorkshire and Meishan pigs by qRT-PCR. The expression level was normalized to β-actin and measured with 2^(-ΔΔCt) value. Results are averaged from three independent replicates during all stages. Error bars represent SD (n = 3). Significant levels were analyzed by t-test. ** = $p<0.01$; * = $p<0.05$

with Meishan pigs. No significant expression differences in Meishan and Yorkshire pigs between 60 and 120 days were observed.

The relative expression level of FABP3 at 60 days is significantly higher ($p<0.05$) in Meishan than Yorkshire pigs but showed no significant difference at other stages between the two breeds.

Differential expression of FABP4 during porcine skeletal muscle development between breeds: Porcine FABP4 was expressed at 65 dpc with a very low level then increased to a peak at 3 days and was down-regulated from the 3 days to the 120 days in Meishan pigs ($p<0.05$). In Yorkshire pigs, the expression trend of FABP4 from the 65 dpc to the 3 days is generally coincident with Meishan. However, there is no expression difference between 3, 60 and 120 days. The relative expression level of FABP4 was significantly higher ($p<0.05$) in Meishan than Yorkshire pigs at three postnatal development stages and no significant expression differences at 65 dpc between two breeds (Fig. 2).

In this study, researchers characterized the regulation of FABP3 and FABP4 mRNA during porcine skeletal muscle development between two breeds by real-time reverse transcription-PCR. This report is the first to quantitatively compare FABP3 and FABP4 mRNA between Yorkshire and Meishan pigs and laid the basis for future research on *FABP3* and *FABP4* genes as the model genes to study mechanisms of intramuscular fat deposit. The temporal expression data indicates that the mRNA expression levels of porcine FABP3 and FABP4 are highest at postnatal 3 days and up-regulated during skeletal muscle postnatal development. Researchers infer that pig *FABP3* and *FABP4* genes were involved in fatness deposition and intramuscular fat content this relationship is most likely a consequence of higher fatty acid metabolism during skeletal muscle postnatal development. In previous study, significant relationship between FABP3 and FABP4 mRNA expression levels and intramuscular fat content were found in pig (Damon *et al*., 2006; Reiter *et al*., 2007).

Researchers also showed that the expression of FABP3 and FABP4 in Meishan pigs was higher than that in Yorkshire pigs at postnatal development stages. In previous study, FABP3 and FABP4 mRNA was induced during adipogenic differentiation in isolated adipocytes from subcutaneous adipose tissue or muscle in growing pigs (Gardan *et al*., 2007; Li *et al*., 2007; Samulin *et al*., 2008). Chinese fat-type Meishan pig breed and the lean-type Yorkshire pig breed show significant differences in muscle growth and meat quality traits. Yorkshire pigs are well known for their high growth rate and lean meat percentage whereas Meishan pigs have low growth rate and low lean meat percentage but have good meat flavor.

CONCLUSION

This study shows that the expression profile of porcine FABP3 and FABP4 indicated that they may have an important role in the development of intramuscular adipocytes.

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