

Expression and polymorphism of genes related to intramuscular fat content in pigs

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Introduction

Intramuscular (i.m.) fat, visible as marbling in meat, is one of the most important factors for meat quality. In the past decades, the aim of pig production in developed countries and also in China was directed to increase the lean percentage of carcass. This effort resulted in a decreased i.m. fat content. Pork becomes tougher and less flavouring. There has been many endeavors to raise pork quality, which were focused also on i.m. fat. It is difficult to measure i.m. fat content precisely in living pigs. Consequently, it is important to develop effective markers for i.m. fat content. In cattle Barendse (1999) considered thyroglobulin, and Thaller et al. (2003) DGAT 1 as a positional and functional candidate gene for i.m. fat deposition. Schmutz et al. (2002) found that genetic variants of leptin had effects on meat quality. In pigs Gerbens et al. (1998, 1999) studied the effects of genetic variants of heart fatty acid binding protein (HFABP) and adipocyte fatty acid binding protein (AFABP) genes on intramuscular fat. A HFABP RFLP marker and AFABP microsatellite marker were found to be related to i.m. fat content. Our research intended to develop some more markers for i.m. fat content in Chinese pig breeds. Erhualian is a local line of Taihu pigs, a Chinese local breed which is most famous for its high fertility. Nevertheless, the growth rate and lean percentage of Erhualian pigs is poorly low. Sutai is a newly fostered breed in China which possesses half Duroc and half Taihu pigs. The growth rate and lean percentage are raised considerably in Sutai pigs compared to Erhualian pigs. Both Erhualian and Sutai pigs possess excellent meat quality which characterized by high percentage of i.m. fat. In this study, these two breeds were used in three experiments.

Material and Methods

In experiment I 60 Sutai fattening pigs were used for detecting correlation between i.m. fat content and expression level of candidate genes. Samples were collected from longissimus

muscle 30 minutes after slaughter. Intramuscular fat content was determined by Soxhlet petroleum-ether extraction. Expression level was measured for HFABP, AFABP, cAMP responsive element binding protein (CREB), adipocyte determination and differentiation-dependent factor 1 (ADD1), fatty acid synthase (FAS), and hormone sensitive lipase (HSL) by means of semi-quantitative RT-PCR. 18S rRNA was used as internal standard. Correlations were calculated by SPSS 11.0 software. In experiment II the genes correlated to i.m. fat was studied during growth in Erhualian pigs. At 3, 20, 45, 90, 120 and 180 d of age longissimus muscle were collected after slaughter. In each age group, there were 8 individuals. One-way ANOVA was applied to detect differences between age groups. In experiment III PCR-SSCPs method was used to find polymorphism in candidate genes correlated with i.m. fat. Longissimus muscle were collected from 100 Sutai fattening pigs. I.m. fat content and back fat thickness of different genotype was compared to each other by One-way ANOVA. For genes with different i.m. fat between genotypes nucleotide sequencing were done for each allele. The significance level for all experiments was $P < 0.05$.

Results and Discussion

Experiment I: The mRNA level of AFABP, ADD1, and the ratio of FAS to HSL genes were correlated with i.m. fat in longissimus muscle in Sutai pigs, but HFABP, CREB, FAS and HSL were not (Tab.1). The physiological effects of AFABP is to accelerate FA absorption by cells as well as FA diffusion in cells (Peeters et al., 1989; Glatz and Van Der Vusse, 1996), which facilitate triglyceride synthesis in cells. The higher AFABP level is, the more fat will accrete in cells. ADD1 is one of the most important transcription factors in adipocyte differentiation. It is reported that ADD1 promotes preadipocytes differentiation by activating PPAR γ expression (Kim and Spiegelmann, 1996; Kim et al., 1998). A higher ADD1 level result in a higher adipocyte differentiation as well as more fat cells in muscle. FAS and HSL are rate-limiting enzymes respectively in triglyceride synthesis and degradation. In our study, we found no correlation between i.m. fat content and FAS or HSL mRNA level. However, a moderate correlation was found between i.m. fat content and mRNA ratio of FAS to HSL. This result indicated that the amount of i.m. fat depot was affected by both synthesis and degradation processes. The ratio may reflect the ability of i.m. fat deposition.

Tab. 1: Correlation between candidate genes mRNA level and i.m. fat content

	HFABP	AFABP	CREB	ADD1	FAS	HSL	FAS/HSL
i.m. fat (%)	0	0.63	0	0.68	0	0	0.46

Experiment II: For the genes which were found to be correlated with i.m. fat, we investigated their expression pattern during growth in Erhualian pigs. During growth it is known that i.m. fat deposition increased. In our study ADD1 expression level increased after 90 d, which corresponded to the beginning of sexual development in Erhualian pigs (Fig.1). The ratio of FAS to HSL increased after 120 d, mainly due to the decreasing of HSL expression level (Fig.2). This is in accordance with the function of these genes in adipocyte development. However AFABP expression level declined from birth to weaning, and after weaning was no change (Fig.1). The result was unexpected but similar to the study of Ding et al. (1999), which showed that during 0-28 d, the mRNA level of AFABP was highest on 3 d, and after that it decreased.

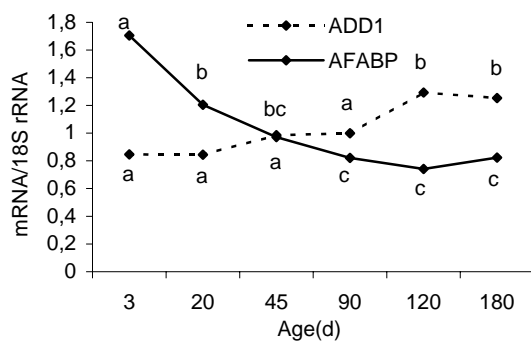


Fig. 1. ADD1 and AFABP expression pattern during growth in Erhualian pigs

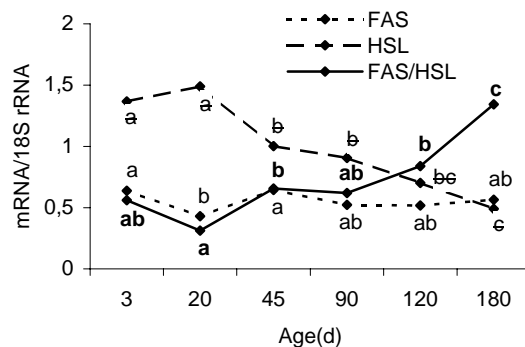


Fig. 2. FAS, HSL expression pattern and FAS/HSL during growth in Erhualian pigs

Experiment III: A polymorphism site was found in ADD1 gene locus by means of PCR-SSCPs method. Mutations on genes involved in i.m. fat deposition are more likely to be effective on i.m. fat content, especially when the mutation are in exons. In our study, nucleotide sequencing analysis revealed that there were two C/T substitution sites at 347th and 374th bp on ADD1 gene respectively. Both mutations were found in exon. For A and B alleles, the nucleotide sequences were -T-C- and -C-T- respectively. I.m. fat content was higher in AB genotype compared to AA. However, there was no difference in i.m. fat content between two homozygotes. Additionally, back fat thickness had no difference among three genotypes. The effect appeared to be independent of backfat and so offers great promise for the manipulation of this trait by Marker Assisted Selection. Further research is needed to elucidate if this PCR-SSCPs marker can be used as an index for i.m. fat selection.

Tab. 2: i.m. fat content and backfat thickness of three genotypes on ADD1 locus

Genotype	n	i.m. fat (%)	Back fat thickness (cm)
AA	50	8.0 ± 2.9 ^a	2.5 ± 0.6 ^a
AB	36	10.6 ± 3.4 ^b	2.6 ± 0.5 ^a
BB	14	9.5 ± 2.4 ^{ab}	2.5 ± 0.6 ^a

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