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Gene Expression Changes in Porcine AdipoQ and its Receptors, AdipoR1 and R2 in Adipose Tissues

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Abstract: The three adiponectin-related genes (*AdipoQ*, *AdipoR1*, *AdipoR2*) have been notably identified in association with adiponectin levels *in vivo* and obesity phenotypes. The mRNA levels of AdipoQ, AdipoR1 and AdipoR2 have been measured across six different adipose tissues from the female leaner Landrace and female fatty Rongchang pig breeds using quantitative real time RT-PCR (q-PCR) approach. The mRNA levels of AdipoQ ($P_B = 2.27\times10^{-9}$), AdipoR1 ($P_B = 0.04$) and AdipoR2 ($P_B = 5.72\times10^{-6}$) were higher in the leaner Landrace pigs than in the fatty Rongchang pigs. The mRNA levels of AdipoQ ($P_{T\,(VATs\,vs.\,SATs)} = 2.67\times10^{-3}$) and AdipoR2 ($P_{T\,(VATs\,vs.\,SATs)} = 2.27\times10^{-4}$) were higher in SATs compared with VATs. These results present the breed and tissue-specific expression patterns of AdipoQ, AdipoR1 and AdipoR2 which highlight their potential as candidate genes for the pig fat mass trait.

Key words: Pig, AdipoQ, AdipoR1, AdipoR2, adipose, qRT-PCR, China

INTRODUCTION

Adipose tissue is recognized as an organ that not only stores energy but also acts as a multifunctional endocrine tissue (Schaffler and Scholmerich 2010). Adipose tissue secretes a variety of bioactive, factors (termed adipokines) that regulate systemic processes including food intake, nutrient metabolism, insulin sensitivity, stress responses, reproduction, bone growth and inflammation (Ouchi *et al*., 2011). Adiponectin (AdipoQ) an adipocyte-specific adipokine has attracted attention because it exerts beneficial pleiotropic effects on many obesity-related diseases (Lancaster and Febbraio 2011). AdipoQ can inhibit the synthesis of malonyl-CoA via the cell-surface receptors AdipoR1 and R2 resulting in the increase of mitochondrial import and fatty acid oxidation (Lago *et al*., 2009).

Previous reports indicated that the adipose tissues in different body sites are functionally and metabolically distinct (Ibrahim, 2010). The Visceral Adipose Tissues (VATs) are more directly related to obesity-related diseases compared with the Subcutaneous Adipose Tissues (SATs) (Fox *et al*., 2007; Sam *et al*., 2008). VATs are considered to have greater insulin-resistance compared with SATs and preferential access to the liver through the portal vein, thereby providing free fatty acid as a substrate for hepatic lipoprotein metabolism and glucose production (Sam *et al*., 2008; Cao, 2010). In this study, researchers measured the breed and tissue-specific expression patterns of the *AdipoQ*, *AdipoR1* and *AdipoR2* genes across six adipose tissues from different body areas of the fatty Rongchang (Chinese breed) and the lean Landrace (Western breed) pigs using quantitative RT-PCR (qRT-PCR).

Researchers demonstrate that the mRNA levels of AdipoQ and AdipoR2 in adipose tissues were negatively associated with adipose deposition. Nonetheless, AdipoR1 was less affected by the intrinsic differences between VATs and SATs.

MATERIALS AND METHODS

Animals and tissue collection: Six different adipose tissues were collected from nine female Rongchang and 9 female Landrace pigs at the age of 210 days. The Greater Omentum (GOM), Mesenteric Adipose (MAD) and Retroperitoneal Adipose (RAD) tissues are located within the abdominal cavity and are known as VATs. The Abdominal Subcutaneous Adipose (ASA) and the Upper (ULB) and Inner Layers of the Backfat (ILB) tissues are

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Table 1: Primers used for qRT-PCR analysis

Gene symbol	Sequences of primers (5'→3')	Amplicon length (bp)	GenBank no.
AdipoQ	F: GGGTCACTGTCCCTAAC	228	NM_214370
	R: GTCCTGGTACTGGTCGT		
AdipoR1	F: CGAGGTGGTCAAGGCTAAG	101	NM_001007193
	R: CAATGGCGTGGAGAAATAC		
AdipoR2	F: CCTCTTACAAGCCCACC	107	NM_001007192
	R: AGTCAGGCAGCACATCG		
ACTB	F: TCTGGCACCACACCTTCT	114	DQ178122
	R: TGATCTGGGTCATCTTCTCAC		
TBP	F: GATGGACGTTCGGTTTAGG	124	DQ178129
	R: AGCAGCACAGTACGAGCAA		
TOP2B	F: AACTGGATGATGCTAATGATGCT	137	AF222921
	R: TGGAAAAACTCCGTATCTGTCTC		

β-actin (ACTB), TATA Box Binding Protein (TBP) and Topoisomerase IIβ (TOP2B) were used as internal control genes

the typical SATs. Pigs were allowed access to feed and water *ad libitum* under the same normal conditions and were humanely sacrificed as necessary to ameliorate suffering.

RNA extraction and qRT-PCR: Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The qRT-PCR was performed using the SYBR Green PCR kit (TaKaRa, Dalian, Liaoning, China) on an iQ5 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. The primers for the three target genes (*AdipoQ*, *AdipoR1* and *AdipoR2*) and the three housekeeping genes (ACTB, TBP and TOP2B) are shown in Table 1 (Erkens *et al*., 2006).

Data analysis: The $2^{-\Delta\Delta Ct}$ method was used to determine the relative mRNA expression changes between surveyed samples. The normalization factors of the three housekeeping genes and the relative quantities of the target genes were analyzed using the qBase software (Bio-Rad, Hercules, CA, USA) (Hellemans *et al*., 2007). Statistical analysis was performed using the SigmaPlot 12.0 software (Systat, San Jose, CA, USA).

RESULTS AND DISCUSSION

The Landrace pigs had a higher body weight compared with the Rongchang pigs at the age of 210 days (1.22 fold, $p = 1.05\times10^{-3}$; Fig. 1a). However, the Rongchang pigs exhibited a higher backfat thickness compared with the Landrace pigs (2.02 fold, $p = 2.99\times10^{-7}$; Fig. 1b). This result is consistent with their breeding history in which the lean Landrace pigs have been continuously selected for muscle growth whereas the fatty Rongchang pigs have been selected for higher adipose deposition.

The mRNA levels of AdipoQ ($P_B = 2.27\times10^{-9}$), AdipoR1 ($p_B = 0.04$) and AdipoR2 ($p_B = 5.72\times10^{-6}$) were higher in the lean Landrace pigs compared with the fatty

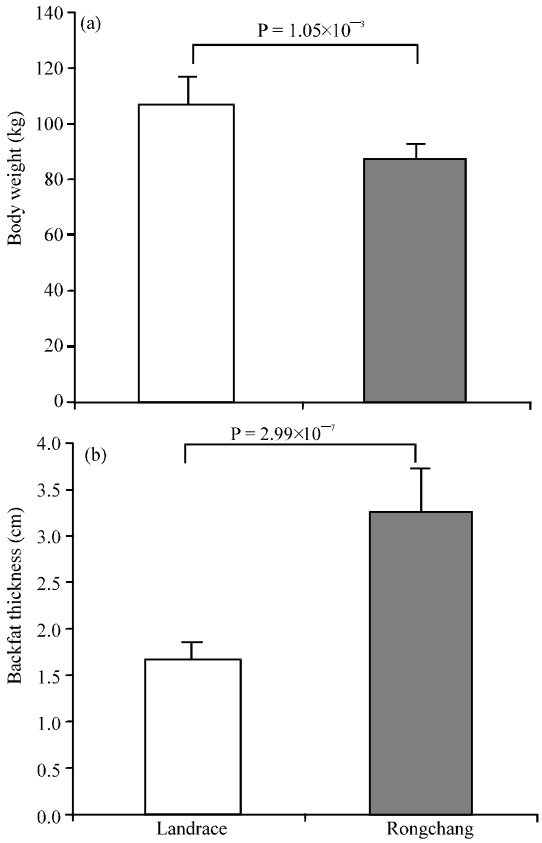

Fig. 1: a) Differences in body weight b) and backfat thickness between Landrace and Rongchang pigs. Student t-test, n = 9. Values are means±SD

Rongchang pigs in all six adipose tissues (Fig. 2). This result is consistent with the biological roles of AdipoQ which can increase fatty acid oxidation and reduce glucose synthesis via the AdipoR1 and R2 receptors (Lago *et al*., 2009; Ikeoka *et al*., 2010). The levels of circulating AdipoQ and its receptors in the plasma are reduced in morbidly obese patients but increased during the process of weight loss (Maeda *et al*., 2001; Gomez-Abellan *et al*., 2010).

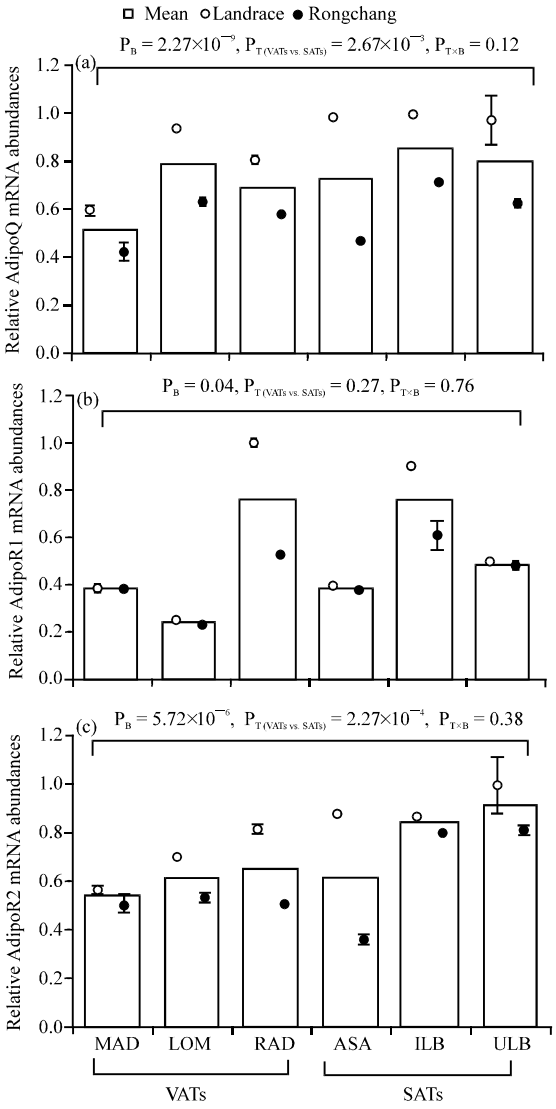

Fig. 2: a) Breed and tissue specific mRNA expression patterns for AdipoQ, b) AdipoR1 and c) AdipoR2 across six adipose tissues in Landrace and Rongchang pigs. Two-way repeated-measures ANOVA, n = 9. B and T stand for breed and tissue, respectively. Values are means±SD

The mRNA levels of AdipoQ ($P_{T\ (VATs\ vs.\ SATs)} = 2.67\times10^{-3}$) and AdipoR2 ($P_{T\,(VATs\,vs.\,SATs)} = 2.27\times10^{-4}$) were higher in SATs (ASA, ULB and ILB) compared with VATs (GOM, LOM and RAD; Fig. 2a and c) which can be explained by the fact that VATs are more metabolically active and insulin-resistant than SATs (Frayn, 2000). Previous *in vitro* and *in vivo* research also indicated that the down-regulation of AdipoQ production was associated with decreased expression of AdipoR2 (but not of AdipoR1) (Lihn *et al*., 2004; Nannipieri *et al*., 2007).

Notably, unlike AdipoQ and AdipoR2, the differences in AdipoR1 mRNA levels ($P_{T\,(VATs\ vs.\ SATs)} = 0.27$) between VATs and SATs were not statistically significant (Fig. 2b). Previous reports suggested that in contrast to AdipoR1, the mRNA levels of AdipoR2 had a strong negative correlation with the mass of body fat in female pigs (Lord *et al*., 2005). In addition, tumor necrosis factor-α can down-regulate the mRNA levels of AdipoQ and AdipoR2 in cultured stromal-vascular cells but not of AdipoR1 (Lord *et al*., 2005). This result suggests that the mRNA level of AdipoR1 is less affected by the intrinsic functional and metabolic differences between VATs and SATs.

CONCLUSION

In this study, the results suggest that AdipoQ and AdipoR2 are potentially important for porcine adipose deposition and are promising candidate genes for pig fat mass regulation.

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