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Short Communication

Differential expression analysis of porcine *MDH1*, *MDH2* and *ME1* genes in adipose tissues

S.L. Zhou[1]*, M.Z. Li[1]*, Q.H. Li[2], J.Q. Guan[1] and X.W. Li[1]

[1]Institute of Animal Genetics and Breeding,
College of Animal Science and Technology,
Sichuan Agricultural University, Sichuan, China
[2]Hangzhou Academy of Agricultural Sciences, Zhejiang, China

*These authors contributed equally to this study.
Corresponding author: X.W. Li
E-mail: lixuewei9125@126.com

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ABSTRACT. Malate dehydrogenases 1 and 2 (*MDH1* and *MDH2*), and malic enzyme 1 (*ME1*) play important roles in the Krebs cycle for energy metabolism. The mRNA abundance changes of *MDH1*, *MDH2* and *ME1* genes were measured across six different adipose tissues from the leaner Landrace and fatty Rongchang pig breeds using quantitative real-time PCR. The mRNA of *MDH1*, *MDH2* and *ME1* was more abundant in fatty Rongchang pigs than in leaner Landrace pigs. In both breeds, females exhibited higher adipocyte volume and mRNA abundance of *MDH1*, *MDH2* and *ME1* compared with males. These values were higher in the subcutaneous adipose tissue compared with visceral adipose tissue. Furthermore, mRNA abundance changes of *MDH1*, *MDH2* and *ME1* have the remarked significant positive correlation with adipocyte volume across the six adipose tissue types. We conclude that there are breed-, gender- and tissue-specific expression patterns of *ME1*, *MDH1* and *MDH2*, which highlight their potential as candidate genes for selecting for fat volume in pigs.

Key words: Pig; *MDH1*; *MDH2*; *ME1*; mRNA; Adipose; qRT-PCR

INTRODUCTION

The two essential malate dehydrogenases, *MDH1* (cytosolic malate dehydrogenase) and *MDH2* (mitochondrial malate dehydrogenase), play important roles in the Krebs cycle for energy production through aerobic respiration. *MDH1* and *MDH2* are involved in *de novo* lipid synthesis by providing extramitochondrial reducing equivalents from the oxidation of malate (Schmid et al., 2004; Bourneuf et al., 2006). *ME1* (malic enzyme), an NADP-dependent lipogenic enzyme, is involved in the conversion of L-malate to pyruvate. *ME1* is part of a shuttle that transfers acetyl groups from the mitochondria to the cytosol, forming a link between the glycolytic pathway and the citric acid cycle (MacDonald, 1995). *ME1* promotes the release of acetyl-coenzyme A and NADPH from the mitochondria into the cytosol for *de novo* fatty acid biosynthesis. In addition, previous studies have indicated that *MDH1*, *MDH2* and *ME1* are associated with type II diabetes (Schmid et al., 2004; Zhong et al., 2010), and the synthesis and secretion of lipids (Bourneuf et al., 2006).

The adipose tissue located within the abdominal cavity (Ibrahim, 2010), known as visceral adipose tissue (VAT), has been suggested to be anatomically, functionally and metabolically distinct from the subcutaneous adipose tissue (SAT). Currently, there is considerable interest in VAT, due to its relationship with various diseases such as cardiovascular disease, type II diabetes, hyperlipidemia, and other metabolic syndromes. There are a number of potential reasons why VAT within the abdominal cavity may contribute to abnormalities in metabolism; among these are its anatomical site and pattern of venous drainage, and the presence of intrinsic and unique features of visceral adipocytes. The venous drainage of VAT is via the portal system, directly providing free fatty acids as substrates for hepatic lipoprotein metabolism and glucose production.

To survey the breed-, gender- and tissue-specific expression patterns of the *MDH1*, *MDH2* and *ME1* genes, we measured the mRNA abundance levels across six adipose tissues obtained from different body sites of fatty and leaner pig breeds.

MATERIAL AND METHODS

Animals and tissue collection

Six different adipose tissues were collected from nine male and nine female Rongchang (fatty, Chinese breed) and Landrace (leaner, Western breed) pigs at 210 days old. The greater omentum (GOM), the lesser omentum (LOM) and retroperitoneal adipose (RAD) are located within the abdominal cavity, known as VATs. The abdominal subcutaneous adipose (ASA), upper layer of backfat (ULB) and inner layer of backfat (ILB) are the typical SATs. In total, 36 pigs were used in this study. Pigs were allowed access to feed and water *ad libitum* under the same normal conditions and were humanely sacrificed as necessary to minimize suffering.

Adipocyte volume

After being sacrificed, all adipose tissues were fixed in 10% neutral buffered formalin solution, embedded in paraffin using TP1020 semi-enclosed tissue processor (Leica, Germany), sliced at a thickness of 6 μm using an RM2135 rotary microtome (Leica) and stained with

hematoxylin and eosin. The mean diameter of an adipocyte cell was calculated by the geometric average of the maximal and minimal diameter, and 100 cells were measured for each sample in randomly selected fields using a TE2000 fluorescence microscope (Nikon, Japan) and the Image Pro-Plus 6.0 software (Media-Cybernetics, USA) (Li et al., 2008).

RNA extraction and reverse transcription

Total RNA was extracted by RNAiso plus (TaKaRa, China), and the cDNA template reverse transcription (RT) reaction was reversed using random 6 mers and OligodT primer of the PrimeScript RT reagent kit with gDNA Eraser (TaKaRa), according to the manufacturer instructions.

Quantitative real-time RT-PCR (q-PCR)

q-PCR was performed using the SYBR Green PCR kit (TaKaRa) on an iQ5 Real-Time PCR Detection System (Bio-Rad, USA). Each reaction comprised 12.5 μL SYBR Green q-PCR Super Mix, 2 μL cDNA, 10 pmol of each primer and RNA-free water to a total volume of 25 μL. The real-time PCR program started with a 3-min denaturation at 95°C, followed by 40 cycles of 15 s denaturation at 95°C and 30 s annealing⁄elongation at the annealing temperature for each specific primer, during which fluorescence was measured. The specific PCR products were confirmed by melting curve analysis; this allowed the verification of the presence of one gene-specific peak and the absence of primer dimers. Table 1 lists the primers of three objective genes (i.e., *MDH1*, *MDH2* and *ME1*) that were designed by using the Primer 5.0 software and three housekeeping genes (i.e., *ACTB*, *TBP* and *TOP2B*) (Erkens et al., 2006).

Table 1. Information on the primers.

Gene symbol	Sequences of primers (5'→3')	Amplicon length (bp)	GenBank accession No.
MDH1	F: TAAGGTTATCGTGGTGGG R: TGCTTTAGCTCGGTTGTG	124	U44846
MDH2	F: CGAGGTGGTCAAGGCTAAG R: CAATGGCGTGGAGAAATAC	172	M16427
ME1	F: GTTGCCCTTGGTGTTGT R: GGATAAATGGTGGCTGTC	212	X93016
*ACTB**	F: TCTGGCACCACACCTTCT R: TGATCTGGGTCATCTTCTCAC	114	DQ178122
*TBP**	F: GATGGACGTTCGGTTTAGG R: AGCAGCACAGTACGAGCAA	124	DQ178129
*TOP2B**	F: AACTGGATGATGCTAATGATGCT R: TGGAAAAACTCCGTATCTGTCTC	137	AF222921

*β actin (*ACTB*), TATA box-binding protein (*TBP*) and topoisomerase II β (*TOP2B*) are the housekeeping genes.

Data analysis

The $2^{-\Delta\Delta Ct}$ method was used to determine the relative mRNA abundance for the surveyed samples. All measurements contained a negative control (i.e., no cDNA template), and each sample was analyzed in triplicate. Normalized factors of three housekeeping genes and relative quantities of objective genes were analyzed using the qBase software (Hellemans et

al., 2007). We examined nine individual pigs for each breed per gender and used three-way repeated-measures ANOVA for determining the significance of the differences using Sigma Plot 12.0 (Systat Software Inc., USA). The Pearson correlation test was used to determine the relationship between the mRNA abundance and adipocyte volume over six adipose tissues.

RESULTS AND DISCUSSION

Between the two pig breeds, the Rongchang pigs had a higher adipocyte volume (~251.48 × 10^3 μm^3) than the Landrace pigs (~157.35 × 10^3 μm^3; P = 1.19 × 10^{-8}; Figure 1), which is consistent with their breeding history. The Landrace pigs have been continuously selected for less adipose, whereas the Rongchang pigs have been selected for higher adipose deposition ability. Similar to previous reports, *MDH1* (P = 1.44 × 10^{-10}), *MDH2* (P = 1 × 10^{-15}) and *ME1* (P < 1 × 10^{-16}) exhibited higher mRNA abundances in the fatty Rongchang breed compared to the leaner Landrace breed (Figure 2), which is consistent with the biological function of these genes, promoting adipose deposition (Webb et al., 1973; Schmid et al., 2004; Bourneuf et al., 2006). It has been demonstrated by two-dimensional electrophoresis that MDH1 protein levels are upregulated in rats placed on a high-fat diet (Choi et al., 2010). Also, the mRNA abundance of *MDH2* in the liver is upregulated in fatty versus leaner chickens (P = 0.03) (Bourneuf et al., 2006). Furthermore, low-fat-fed rats exhibit lower *ME1* activity compared to normal controls (Draznin et al., 1989).

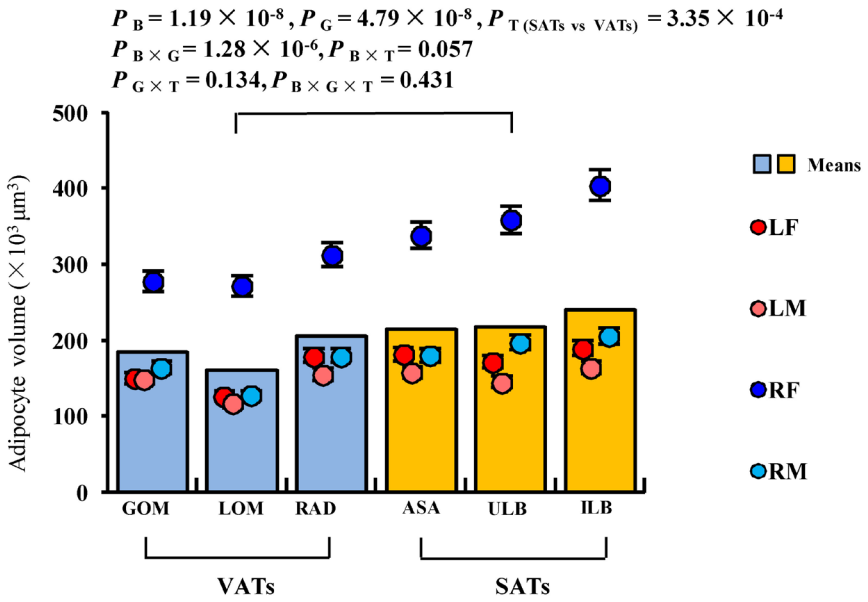

Figure 1. Adipocyte volume differences between breeds, genders and tissues. Breed = L and R represent the Landrace and Rongchang pigs, respectively. Genders = M and F represent male and female, respectively. P values were derived from three-way repeated-measures ANOVA. B, G and T stand for breed, gender and tissue, respectively. SAT = subcutaneous adipose tissue; VAT = visceral adipose tissue; GOM = greater omentum; LOM = lesser omentum; RAD = retroperitoneal adipose; ASA = abdominal subcutaneous adipose; ULB = upper layer of backfat; ILB = inner layer of backfat. Values are reported as means ± SD.

Between genders, females (~247.01 × 10^3 μm^3) exhibited higher adipocyte volumes compared to males (~161.82 × 10^3 μm^3; P = 4.79 × 10^{-8}) in both breeds (Figure 1). These phenotypic differences are consistent with the mRNA abundance differences between males and females. As shown in Figure 2, *MDH1* (P = 0.437), *MDH2* (P = 1.37 × 10^{-3}) and *ME1* (P = 5.16 × 10^{-4}) exhibited higher mRNA abundance in females compared to males, indicating that the adult females are fatter than the males. We speculate that male pigs are more aggressive and active, thus expend more energy than females when reaching sexual maturity at 210 days.

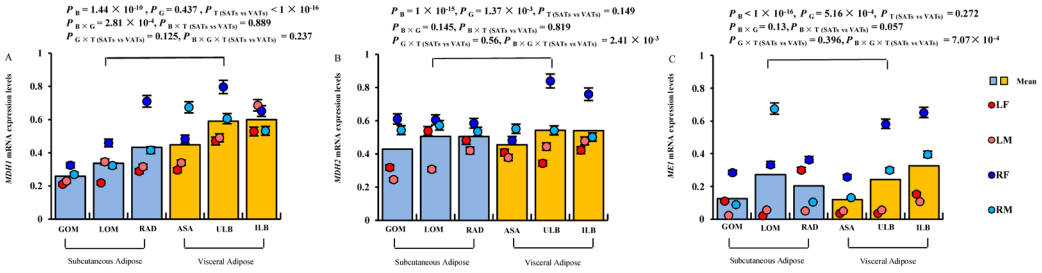

Figure 2. Breed-, gender- and tissue-specific gene expression patterns of (**A**) *ME1*, (**B**) *MDH1* and (**C**) *MDH2*. For abbreviations, see legend to Figure 1.

Notably, the three SATs (ASA, ULB, ILB; ~224.55 × 10^3 μm^3) had a higher adipocyte volume than the compartmental VATs (GOM, LOM, RAD; ~182.24 × 10^3 μm^3; P = 3.35 × 10^{-4}; Figure 1). As shown in Figure 2, *MDH1* (P < 1 × 10^{-16}), *MDH2* (P = 0.149) and *ME1* (P = 0.272) also exhibited higher mRNA abundance in SATs compared to VATs, indicating that VATs are more metabolically active, and sensitive to lipolysis than SATs. These differences correspond to the anatomic, functional and metabolic distinctions between VATs and SATs (Ibrahim, 2010). Previous studies have demonstrated that the lipolytic action of catecholamines decreases in SATs but increases in VATs, while the antilipolytic effect of insulin and prostaglandins is much less pronounced in visceral adipocytes compared to subcutaneous adipocytes (Arner, 2002).

Moreover, the expression changes in *MDH1* (r = 0.593, P = 2.24 × 10^{-3}), *MDH2* (r = 0.713, P = 9.18 × 10^{-5}) and *ME1* (r = 0.645, P = 6.59 × 10^{-4}) showed a strong positive correlation with adipocyte volume (Figure 3). This is consistent with previous findings indicating that the ubiquitously expressed *ME1* is positively associated with the deposition of porcine intramuscular adipose (r = 0.9, P < 0.01) (Mourot and Kouba, 1999).

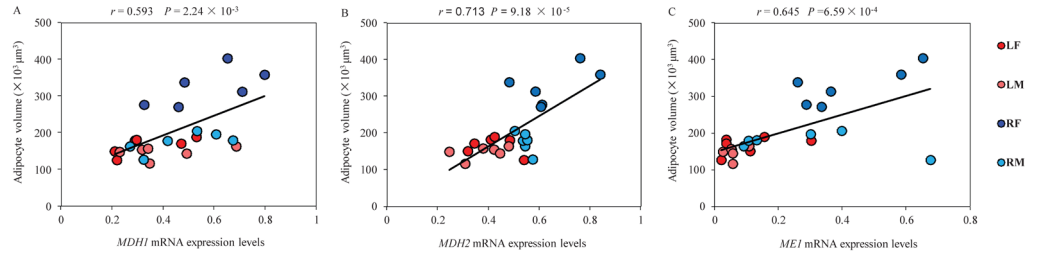

Figure 3. Pearson correlation test between adipocyte volume and mRNA abundance of (**A**) *MDH1*, (**B**) *MDH2* and (**C**) *ME1* across six adipose tissues. For abbreviations, see legend to Figure 1.

In summary, our results suggest that *MDH1*, *MDH2* and *ME1* are important for porcine adipose deposition and are therefore promising candidate genes for the pig fat mass trait.

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