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Comparative analysis on gene expression profiles in cattle subcutaneous fat tissues

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ABSTRACT

Fat related carcass traits are important to the beef industry due to their association with value of the meat. In this study, we attempted to discover the genes that are associated with fat metabolism by identification of differentially expressed genes in subcutaneous adipose tissues of beef steers with different backfat thicknesses. Microarray analysis was performed using a bovine specific oligo-platform containing 8329 probes. In total, 360 differentially expressed genes were identified and their functions were characterized by bioinformatical tools to elucidate molecular pathways. 45 out of 360 differentially expressed genes were found to be involved in 82 KEGG pathways. Validation of 6 selected differentially expressed genes by quantitative real-time PCR revealed correlations between backfat thickness and their expression levels. Our results suggest that expression differences of novel genes and the genes that have been known as genetic markers for fat related traits may be associated with backfat development in beef cattle. Moreover, the gene expression differences were also compared between two cattle crossbreds. The observed different association between the expression of selected genes and breed types suggested that the mechanisms of fat metabolism may differ in response to genotypes.

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1. Introduction

The amount and distribution of fat are among those key factors which influence the carcass and meat quality in beef cattle (Powell and Huffman, 1973; Ritchie et al., 1993; Wheeler et al., 1994; Lozeman et al., 2001), since beef carcasses can be categorized into yield and quality grades based on carcass weight, subcutaneous fat thickness, rib eye area and marbling (Jeremiah, 1996). Therefore breeding for optimal carcass fat is one of the major goals toward better profitability in beef industry.

A number of quantitative trait loci (QTL) studies have shown candidate genes and their locations on chromosomes that may be associated with backfat thickness in cattle (Moore et al., 2003; Li et al., 2004; Wu et al., 2005). Hishikawa et al. (2005) reported gene expression differences in subcutaneous and visceral fat tissues among cattle, mouse and pig, and demonstrated that expression profiles between tissue types were different and fat accumulation mechanisms were different among animal species. Recent studies also demonstrated that fat formation is a complicated biological process that is maintained by unique pathways detected by microarray gene expression analyses using a novel *in vitro* model of cattle adipocytes (Fernyhough et al., 2007, 2008; Taniguchi et al., 2008a,b). The adipocyte biology has been reported to play pivotal roles in regulation of fat metabolism, glucose absorption, energy metabolism and innate immune response in pigs (Jacobi et al., 2006; Gabler et al., 2008). However, the molecular functions of adipocyte in bovine species have not been well studied especially little knowledge is existing to bridge the gap between the phenotype, backfat thickness and function of the genes expressed in subcutaneous adipose tissue of cattle. Therefore, the objectives of this study were to 1) investigate the molecular mechanisms of subcutaneous fat metabolism in beef cattle by identifying differentially expressed genes using bovine specific oligo microarray for simultaneous analysis of gene expression profiling, and 2) to validate selected genes considered to be closely associated with fat development using quantitative real-time PCR (qRT-PCR). Furthermore, the differentially expressed genes were compared between different backfat thickness groups (high vs. low) and crossbreds (Hereford × Aberdeen Angus vs. Charolais×Red Angus).

2. Materials and methods

2.1. Animal sampling

Eight Hereford × Aberdeen Angus (HEAN) and eight Charolais × Red Angus (CHAR) crossbred steers were fed and slaughtered at Lacombe Research Centre, Alberta, Canada. Details of feeding and management are followed as outlined by Basarab et al. (2007). Steers were harvested at the Lacombe Research Centre abattoir when group average ultrasound backfat thickness reached 8 to 9 mm. The animals were

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Table 1
Comparisons of carcass characteristics between two cattle crossbreds (mean ± SD)

Steer traits	Crossbreds		Significance
	HEAN (*N* = 6)	CHAR (*N* = 6)	
Age at slaughter (d)	475.7 ± 17.2	475.3 ± 15.1	*P* = 0.474
ADG (kg/d)	1.6 ± 0.18	1.6 ± 0.2	*P* = 0.382
Slaughter mass (kg)	550.0 ± 16.4	554.4 ± 11.2	*P* = 0.190
Backfat (mm)	10.8 ± 2.9	8.5 ± 3.5	*P* = 0.017
Cutability (%)	56.7 ± 2.7	59.3 ± 3.4	*P* = 0.015
Rib eye area (cm^2)	73.0 ± 5.6	85.8 ± 10.4	*P* = 0.005
Marbling score[a]	473.3 ± 45.0	440.0 ± 32.9	*P* = 0.098

[a] Marbling score is a measure of intramuscular fat: trace marbling or less = 100 to 399 (Canada A quality grade); slight marbling = 400 to 499 (Canada AA quality grade); small to moderate marbling = 500 to 799 (Canada AAA quality grade); slightly abundant or more marbling = 800 to 1100 (Canada Prime).

stunned with a captive bolt and exsanguinated. Weights of the right and left halves of the warm carcass were recorded. After a 24-h chill, carcass cooler data, including cold carcass weight, backfat thickness at the 12th rib (also know as grade fat), longissimus thoracis area at 12–13th rib position (REA), estimated cutability, and marbling score were recorded (Agriculture Canada, 1992, Table 1). Differences in carcass characteristics were compared between crossbreds and backfat thickness groups by *T*-test (Table 2). The subcutaneous adipose tissues were collected immediately after the animal were slaughtered, placed into liquid nitrogen, and stored under −80 °C until further analysis.

2.2. Total RNA extraction and complementary DNA (cDNA) synthesis

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) from the frozen subcutaneous adipose tissues (Chomczynski and Sacchi, 1987). Two micrograms of total RNA was amplified using Amino Allyl MessageAmp™ II aRNA Amplification kit (Ambion, Austin, TX, USA) for microarray and qRT-PCR analysis.

2.3. Microarray experimental design and data analysis

In each crossbred cattle group, three animals each of high or low backfat thickness (6 in total) were selected from eight animals and then, the RNA extracted from the subcutaneous adipose tissues of these animals were used for microarray analysis to compare the gene expression profiles between high and low groups. Bovine specific oligo-DNA microarray used in this study was *Bos taurus* (bovine) AROS™ V1.1 containing 70-mer oligos representing 8329 genes from *Bos taurus* genome (OPERON Biotechnologies, Inc., Huntsville, AL, USA). Gene sequences used for probe design are obtained from TIGR Cattle Gene Index Release 11 (http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=cattle) and GenBank. The amplified RNA (aRNA) was coupled with Cy3 and Cy5 fluorescent dyes (GE Healthcare, Piscataway, NJ, USA) according to the instruction manual. The Cy-labeled probes were hybridized onto the microarray slides customary printed in Microarray and Proteomics Facility, Department of Biological Science, University of Alberta. In the microarray experiments, hybridizations were carried out between differences in both backfat thicknesses and the crossbreds by pooled RNA method. For comparison of the differentially expressed genes associated with backfat thickness, three high (or low) HEAN backfat samples were compared to pooled low (or high) HEAN backfat samples including triplicates dye-swap hybridizations. For CHAR crossbred, the same method was applied. Probes labeled with Cy3 and Cy5 fluorescent dyes were hybridized on the microarray slides at 42 °C for 18 h. Hybridized slide was washed with low stringency buffer (2 × SSC and 0.5% SDS), high stringency buffer (0.5 × SSC and 0.2% SDS) and 0.05 × SSC. Hybridized slides were scanned with 5 micron resolution and their signal intensities were detected by Q-Scan (Genetix, Hampshire, UK). Data analysis of microarray was performed using GeneSifter™ (VizX Labs, Seattle, WA, USA). The background-corrected signal intensity for each spot was normalized by LOWESS and base 2 log-transformed. Differential gene expression profiles within each breed were compared between high and low backfat thicknesses by *T*-test. Additionally, the differences in gene expression between backfat thicknesses and crossbred groups were analyzed using 2-way analysis of variance (2ANOVA) with backfat and breed as the two factors. Criteria on detection of differentially expressed genes were 2-fold or greater in expression level with $P < 0.05$ which was adjusted by the method of Bonferroni correction. Gene ontology was applied to identify cellular and molecular functions of differentially expressed genes. Analyses of pathways for differentially expressed genes were performed by database searches using Kyoto encyclopedia of Genes and Genomes (KEGG: http://www.genome.jp/kegg/).

2.4. Quantitative Real-Time PCR (qRT-PCR) and data analysis

For validation of the identified differentially expressed genes from above microarray analysis, all eight subcutaneous adipose tissue RNA were applied for quantitative real-time PCR (qRT-PCR) assay. Complementary DNA (cDNA) was synthesized from 800 ng total RNA with SuperScript II reverse transcriptase and oligo (dT) priming (Invitrogen). The RNA abundance was measured by TaqMan® Universal PCR Master Mix with gene specific MGB probes labeled with FAM or VIC fluorescent dyes (Applied Biosystems, Foster City, CA, USA). Oligo nucleotide sequences of primers and probes used for qRT-PCR are shown in Table 3. The PCR amplicon of each gene was confirmed to be interrupted by exon-intron boundary. As an internal control for relative gene expression analyses, 18S ribosomal RNA (*18S rRNA*) gene was the best stable reference gene in the qRT-PCR assay detected by TaqMan assay. Each reaction was carried out with triplicates and run in ABI PRISM 7700 Sequence Detection System (SDS) with software version 1.7 (Applied Biosystems). The thermal cycling condition was as follows; 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. Relative expression level for each gene was calculated by the difference of delta CT value of target gene and that of reference gene. Gene expression fold change was calculated by delta-delta CT method

Table 2
Comparisons of carcass characteristics between backfat thicknesses (mean ± SD)

	Backfat thickness (HEAN)		Significance	Backfat thickness (CHAR)		Significance
	Low BF (*N* = 3)	High BF (*N* = 3)		Low BF (*N* = 3)	High BF (*N* = 3)	
Age at slaughter (d)	484.0 ± 22.1	467.3 ± 6.7	*P* = 0.102	479.0 ± 12.1	471.7 ± 19.6	*P* = 0.139
ADG (kg/d)	1.6 ± 0.2	1.7 ± 0.2	*P* = 0.304	1.6 ± 0.2	1.6 ± 0.2	*P* = 0.433
Slaughter mass (kg)	552.7 ± 18.1	548.3 ± 18.1	*P* = 0.418	561.5 ± 8.7	547.3 ± 9.3	*P* = 0.119
Backfat (mm)	8.3 ± 1.2	13.3 ± 0.6	*P* = 0.007	5.7 ± 1.5	11.3 ± 2.1	*P* = 0.046
Cutability (%)	59.0 ± 1.0	54.3 ± 1.2	*P* = 0.030	62.0 ± 2.0	56.7 ± 2.1	*P* = 0.075
Rib eye area (cm^2)	75.3 ± 6.1	70.7 ± 5.0	*P* = 0.096	94.7 ± 5.5	77.0 ± 2.6	*P* = 0.024
Marbling score[a]	473.3 ± 63.5	473.3 ± 32.1	*P* = 0.500	420.0 ± 17.3	460.0 ± 34.6	*P* = 0.135

[a] Same as shown in Table 1.

Table 3
Oligo nucleotide sequences used for qRT-PCR

Gene symbol	Accession	Oligo nucleotide sequence
ADIPOQ	BC140488	Forward primer: CCAATGTACCCATTCGCTTT Reverse primer: GTAGAGTCCCGGAATGTTGC Probe: TGACGGCAGCACTGGCAAGTT
ADFP	BC102211	Forward primer: GGCCAGGAGACCATTTCTCA Reverse primer: CACATTCTTCCTGGCAAGTTCA Probe: TCCATTCCGCCTTCAA
FABP3	BC102153	Forward primer: CACTTGTGCGGGAAATGGTT Reverse primer: TGCAGTGCCATGGGTGAGT Probe: ACGGGAAACTCATTCTG
ACADL	BC123880	Forward primer: GGAGGAGCAAGCATATTCAAATTG Reverse primer: GGCATGACGATATCTGAATGAAGA Probe: ACAGGCCCAGGTTT
DGAT1	BC118146	Forward primer: GGAACTCCGAGTCCATCACCTA Reverse primer: TCTGATGCACCACTTGTGAACA Probe: TCTGGCAGAACTGGAACA
SPP1	BC102632	Forward primer: CTTCTGCCTCTTGGGCATTG Reverse primer: CTGCCAGAACTGGTCGGTTT Probe: CTCCGCCCTTCCAG
18S rRNA	DQ222453	Forward primer: CCGCGGTTCTATTTTGTTGGT Reverse primer: CGGCCGCCCCTCTTAA Probe: TTCGGAACTGAGGCCAT

based on the thinnest backfat sample as a calibrator in each crossbred group (Livak and Schnittgen, 2001). Correlation between gene expression level and backfat thickness was examined by linear regression analysis.

3. Results and discussion

The growth and carcass traits of six beef steers from each crossbred used in this study are shown in Table 1. HEAN had 2.3 mm more ($P<0.05$) backfat over the 12th–13th rib than CHAR, while CHAR had 2.6 percentile points higher estimated cutability ($P<0.05$) and 12.8 cm^2 more REA ($P<0.05$) than HEAN. Both crossbreds were similar in age at slaughter, ADG during the finishing period, slaughter weight and marbling score. Differences in carcass traits between these two crossbreds may be due to the genetic components of HEAN and CHAR. For example, British breeds such as Hereford (genetic component in HEAN) and Angus are known to have characteristic of earlier maturing, earlier fattening, while continental breeds such as Charolais (genetic component in CHAR) are known to be later maturing, later fattening (Berg and Butterfield, 1976; Marshall, 1994).

Steers within each crossbred group were chosen for their formed two distinct backfat thickness groups (Table 2). For example, high backfat HEAN had 5.0 mm more backfat ($P<0.01$) and a lower cutability (4.7 percentile points; $P<0.05$) than low backfat HEAN, while high backfat CHAR had 5.6 mm more backfat ($P<0.05$) and tended to have 5.3 percentile points lower cutability ($P<0.08$) than low backfat CHAR.

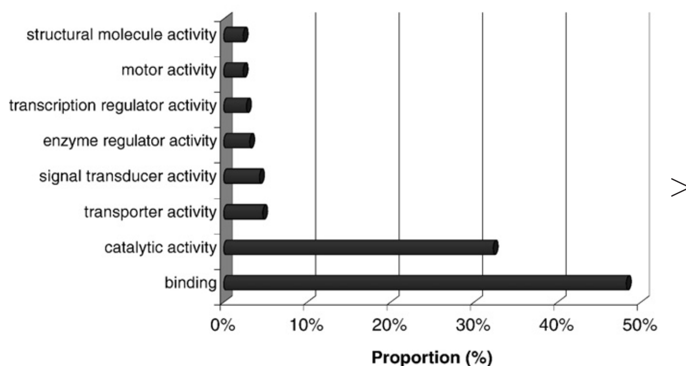

Fig. 1. Ontology analysis of 360 differentially expressed genes in bovine subcutaneous adipose tissue The *x*- and *y*-axes indicate percentages of the differentially expressed genes and molecular functions, respectively.

Low backfat CHAR also had 17.7 cm^2 more l. thoracis area than high backfat CHAR ($P<0.05$), whereas high and low backfat HEAN were similar in REA.

Microarray analysis demonstrated that a total of 360 differentially expressed genes were detected between low and high backfat groups. No differentially expressed genes were detected when two crossbreds were compared by 2ANOVA. The 360 differentially expressed genes were further analyzed for their potential molecular functions (Fig. 1) and pathways involved (Table 4). In the ontology analysis, most of the differentially expressed genes were found in functions of binding (48.2%) and catalytic activity (32.3%), while those belonging to other molecular function were less than 5% (Fig. 1). It is interesting that the classification of molecular functions of these genes identified in the subcutaneous adipose tissues were similar to those obtained from bovine adipocytes differentiation *in vitro* (Tan et al., 2006; Taniguchi et al., 2008a). This suggests that the bovine fat cells may, in general, contain large portion of common gene expression profiles with adipose cell models in terms of the gene ontology results.

From molecular pathway analysis using KEGG database, 45 out of 360 differentially expressed genes were found to be involved

Table 4
Differentially expressed genes between backfat differences

Accession	Gene (symbol)	KEGG pathway	Fold change	
			HEAN	CHAR
BC140488	Adipocyte complement related protein of 30 kDa (*ADIPOQ*)	Adipocytokine signaling PPAR signaling Type II diabetes mellitus	−2.12	−7.12
BC102211	Adipose differentiation-related protein (*ADFP*)	Not identified	+1.02	+4.76
BC123880	Acetyl-Coenzyme A dehydrogenase, long-chain (*ACADL*)	Fatty acid metabolism PPAR signaling	−4.28	−3.03
BC102153	Fatty acid binding protein (muscle and heart) (*FABP3*)	PPAR signaling	+4.14	+3.96
BC102632	Secreted phosphoprotein 1 (*SPP1*)	Cell Communication ECM-receptor interaction	+19.65	+5.80
BC120058.1	C-terminal binding protein 2 (*CTBP2*)	Wnt signaling pathway (3)	−0.10	−2.07
BC105372.1	Glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1) (*GOT1*)	Carbon fixation (9)	+1.09	+2.30
BC122584.1	Hydroxysteroid (17-beta) dehydrogenase 4 (*HSD17B4*)	Fatty acid metabolism (5)	−1.79	−2.10
BC102509.1	Isocitrate dehydrogenase 2 (NADP+), mitochondrial (*IDH2*)	Citrate cycle (TCA cycle) (3)	+1.30	+2.03
BC118361.1	Major histocompatibility complex, class II, DM beta-chain, expressed (*BOLA-DMB*)	Cell adhesion molecules (3)	+3.41	+1.08
BT020798.1	Mitogen-activated protein kinase kinase 6 (*MAP2K6*)	MAPK signaling pathway (4)	+2.39	+1.23
BC103291.1	Nucleoside phosphorylase (*NP*)	Purine metabolism (3)	−2.09	−3.76
BC118371.1	Paraoxonase 2 (*PON2*)	Bisphenol A degradation (2)	−1.42	−2.81
BC151458.1	Presenilin 1 (*PSEN1*)	Wnt signaling pathway (3)	−1.62	−2.32
X12602.1	RAS p21 protein activator (GTPase activating protein) 1 (*RASp21*)	MAPK signaling pathway (2)	+2.27	+1.54
BC123801.1	SMAD family member 2 (*SMAD2*)	Wnt signaling pathway (6)	−1.71	−6.31
BC151330.1	SMAD family member 4 (*SMAD4*)	Wnt signaling pathway (6)	−1.73	−3.81
AJ313183.1	Tight junction protein 1 (*TJP1*)	Tight junction (3)	−1.89	−2.01

Brackets indicate numbers of KEGG pathways in which the differentially expressed genes are included.

in 82 pathways with some of them belonging to multiple pathways (Table 4). Among those genes, 16 are associated with particularly important pathways such as PPAR, adipocytokine, MAPK and Wnt signaling. Their expression changes between high and low backfat groups within each crossbred are summarized in Table 4. The genes involved in these pathways were also identified from our previous study on bovine adipocytes development (Taniguchi et al., 2008b), suggesting that the *in vitro* model system of bovine adipocytes may be applied to study on the gene expression profiling of fat development *in vivo*.

Six genes were then selected qRT-PCR analysis to investigate the correlation between their expression level and backfat thickness. The candidate genes included genes identified from above microarray analysis such as *FABP3*, *ADFP* and multi-functional genes *ADIPOQ*, *ACADL* and *SPP1*. In addition, the relationships of *DGAT1* expression with backfat thickness were also investigated by qRT-PCR since this gene has been well-known candidate for breeding of fat associated traits in both beef and dairy cattle production (Thaller et al., 2003; Schennink et al., 2007). *DGAT1* gene was not detected as significantly differentially expressed gene from our microarray analysis; this could be due to the detection limit of the microarray analysis. We investigated the expression of this gene and the correlation to backfat thickness based on its important molecular function in lipid metabolism and fat development (Wu et al., 2005; Wang et al., 2005; Chang and Chan, 2007) and its functional association has not been studied in beef cattle. The six selected genes showed up- or down-regulations in the microarray reflected tendencies of positive or negative correlation to backfat thickness in the qRT-PCR assay according to the breed types (Table 4, Fig. 2). Some genes showed seven spots of CHAR instead of eight were due to overlapping of multiple samples (Fig. 2).

In consistent with the microarray result showing up-regulations in high backfat animals in both breeds, a positive correlation between *FABP3* expression and backfat thickness in both crossbreds ($P<0.05$) suggests that backfat thickness level may be associated with its gene expression level (Fig. 2). Expression of *ADFP* gene tended to be positively correlated to backfat thickness in HEAN ($R^2=0.36$, $P=0.12$)

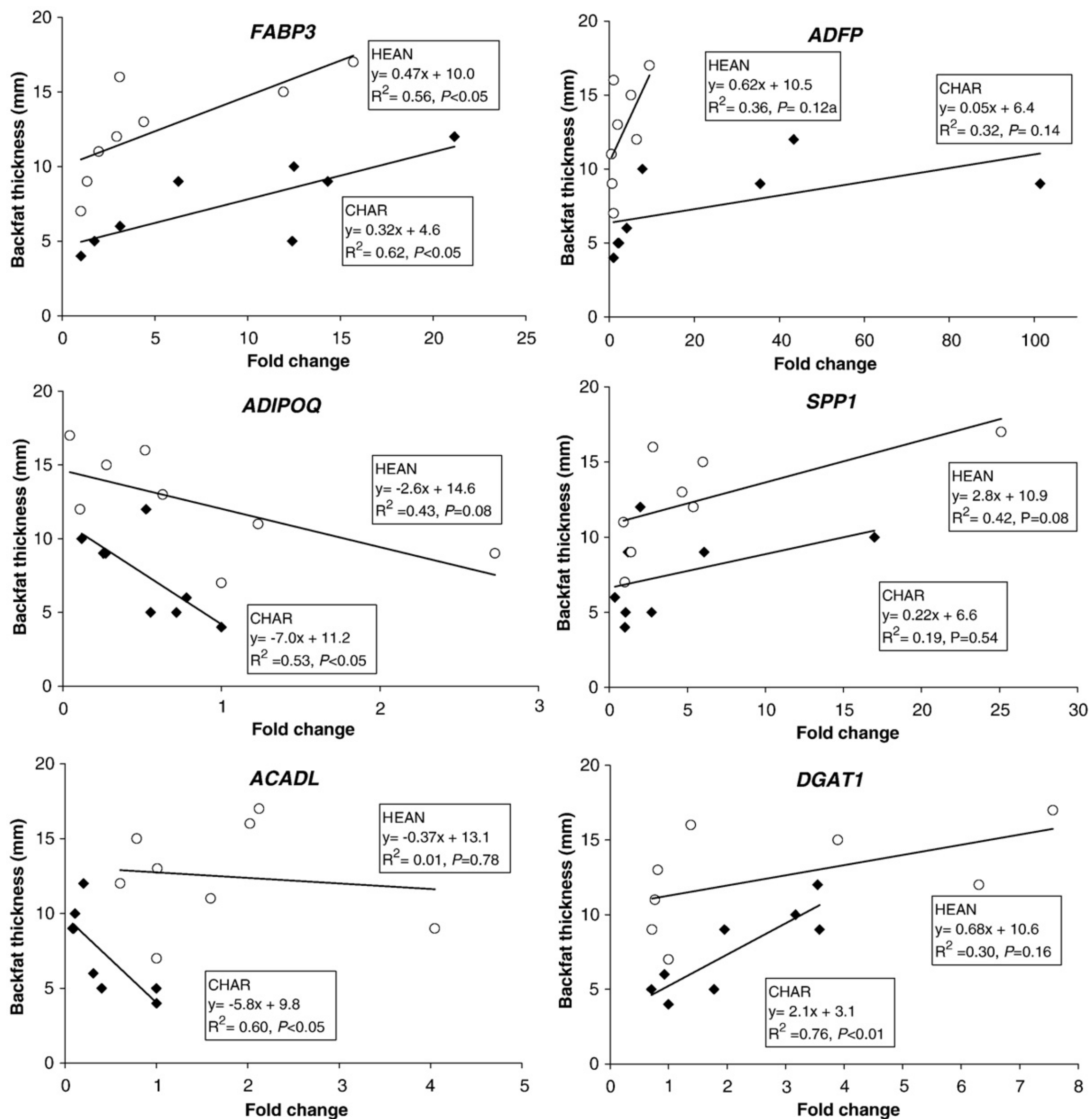

Fig. 2. Correlation between six differentially expressed genes and backfat thickness The *x*- and *y*-axes show fold change of gene expression and backfat thickness, respectively. Opened circles and solid squares show HEAN and CHAR, respectively. In *ADFP*, two CHAR samples having 5 mm backfat thickness were overlapped (2.07-fold and 2.30-fold). In *ADIPOQ*, two CHAR samples having 9 mm backfat thickness were overlapped (0.27-fold and 0.26-fold). In *SPP1*, two samples having 9 mm backfat thickness were overlapped (HEAN: 1.39-fold and CHAR: 1.19-fold). In *ACADL*, two CHAR samples having 9 mm backfat thickness were overlapped (0.08-fold and 0.09-fold).

and in CHAR (R^2=0.32, P=0.14). It has been known that *ADFP* gene encodes a major lipid droplet protein that accumulates lipids and affects lipid homeostasis in mouse adipocytes (Chang and Chan, 2007). Our result suggests that *ADFP* expression may be associated with lipid accumulation which is one of the factors affecting the fat development in bovine subcutaneous fat tissue.

It has been reported that *SPP1* is associated with post-weaning growth in cattle (White et al., 2007), although its function has not been well studied. No association with fat-related carcass traits of this gene has been reported in cattle population. Expression level of *SPP1* gene tended to be positively correlated with backfat thickness in HEAN (P=0.08), whereas no significant correlation was observed in CHAR (Fig. 2). This suggests that *SPP1* gene expression in HEAN may be associated with subcutaneous fat thickness. Similarly, expression level of the *DGAT1* gene was also positively correlated with backfat thickness in CHAR (P<0.01), whereas no significant correlation was observed in HEAN (P=0.16). In cattle breeding, *DGAT1* gene has been used as a genetic marker for fat composition traits such as marbling in German Holstein and Charolais cattle (Thaller et al., 2003) and subcutaneous fat thickness in a Wagyu–Limousin crossbred (Wu et al., 2005). Our previous study revealed that *DGAT1* expression is associated with preadipocyte differentiation in cattle (Taniguchi et al., 2008b). These suggest that *DGAT1* has significant effect on subcutaneous fat development in cattle not only by its genetic polymorphisms (Thaller et al., 2003; Wu et al., 2005), but also by its expression level.

In addition to the positive correlations between gene expression level and backfat thickness, negative correlations were also observed between expression of *ADIPOQ* and *ACADL* genes and backfat thicknesses in CHAR (P<0.05) from both microarray and qRT-PCR analysis in CHAR steers (Table 4). In HEAN, fold change of *ADIPOQ* gene tended to be correlated with backfat thickness (P=0.08), while no significant correlation was observed between *ACADL* expression level and backfat thickness (P=0.78). The expression level of *ADIPOQ* was reported to be negatively associated with adiposity and lipogenesis in pigs (Jacobi et al., 2004), and obesity and insulin resistance in mice (Fruebis et al., 2001; Clarke et al., 2006). The results of *ADIPOQ* expression suggest that *ADIPOQ* is negatively associated with beef subcutaneous fat development in cattle and its effect is more obvious in later fattening (CHAR) than in early fattening breeds or crossbreds (HEAN). Effect of *ACADL* gene on backfat thickness seems to be more significant in later fattening breed crosses (CHAR; R^2=0.60, P<0.05) than that of earlier fattening breed crosses (HEAN; R^2=0.01, P=0.78) (Fig. 2). Our results suggest that the higher expression level of *ACADL* observed in lower backfat CHAR may reflect their higher ratio of energy consumption in the fat tissue (or lower lipid accretion) compared to the fatter CHAR.

The polymorphisms of *SPP1* and *DGAT1* have been evaluated for their associations with beef carcass trait (*SPP1*), beef fat composition, and milk fat composition (*DGAT1*) (Osmundsen et al., 1991; Le et al., 2000; Fruebis et al., 2001; Thaller et al., 2003; Jacobi et al., 2004; Clarke et al., 2006; Schennink et al., 2007; White et al., 2007). The expression differences of *SPP1* and *DGAT1* in subcutaneous adipose tissue suggest that these genes can also be used for association study with backfat thickness. Although the biochemical functions of *ADFP* and *ADIPOQ* genes in *Bos taurus* are not fully understood, the differential expression of *ADFP* and *ADIPOQ* associated with backfat thickness in HEAN and CHAR revealed their potential roles in association studies for subcutaneous fat development.

In this study, the microarray analysis of gene expression profiling characterized molecular functions of 360 differentially expressed genes in mature subcutaneous fat tissue in beef cattle. Validation by qRT-PCR analysis demonstrated relationships between expression levels of six candidate genes and backfat thickness in both crossbreds. CHAR crossbred had more correlations than those of HEAN, suggesting that the effect of those genes might be greater on later fattening crossbreds (CHAR) than earlier fattening crossbreds (HEAN) and/or biological types.

Transcription factors such as PPAR-γ, CCAAT/enhancer binding protein (C/EBP) and sterol element binding protein (SREBP) have been known to be pivotal elements to regulate expression level of adipogenic genes in bovine adipose tissues (Tan et al., 2006; Wang et al., 2005). However, none of them were detected as differentially expressed genes in this study. Bovine PPAR-γ and SREBP were differentially expressed only during the early stage of adipocyte cell development (Taniguchi et al., 2008a), suggesting that subcutaneous fat tissues used in this study might have reached to mature developmental level. The same reason may also account for the lower number of differentially expressed genes detected in this study when compared to our previous study using perimuscular preadipocyte model, which detected >2000 differentially expressed genes during the cell differentiation with the same microarray platform (Taniguchi et al., 2008b). Further study using subcutaneous adipose tissues undergoing developmental stage will be essential to confirm the roles of these transcription factors and to clarify molecular pathway during tissue development in particular in earlier fattening breed such as Hereford and Angus. Our study of gene profiling in bovine adipocyte and subcutaneous fat tissues will supply fundamental knowledge to understand fat metabolisms in cattle as well as other meat producing livestock species.

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