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IDENTIFICATION OF DIFFERENTIALLY EXPRESSED TRANSCRIPTS IN M. *LONGISSIMUS DORSI* WITH DIVERGENT MARBLING PHENOTYPES IN HANWOO (KOREAN NATIVE CATTLE)

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SUMMARY

Marbling is a major trait in determining profit in the Korean beef industry. However, the underlying biology of muscles with divergent marbling phenotypes is still poorly understood in cattle. In this study, we attempted to detect differentially expressed genes in *M. longissimus dorsi* in Hanwoo steers with high and low estimated breeding values for marbling score using an Affymetrix bovine gene expression array. Three data-processing methods (MAS5.0, GCRMA and RMA) were implemented to test for differential expression (DE). Analysis identified 21 transcripts exhibiting significant DE in at least two data-processing methods ($P<0.001$). Squalene epoxidase and Cytochrome P450 gene, thought to play a role in biotransformation of steroids, were expressed more in highly marbled muscle whereas gene for Arginyl-tRNA synthetase, Ribosomal protein S6 kinase, Thimetoligopeptidase 1, Proteosome activator subunit 4, ATP binding protein and CDC-like kinase gene, which are all involved in protein synthesis and cell division, were down-regulated in highly marbled muscle. These results suggest that down-regulation of genes involved in cell division, growth regulation and protein synthesis may lead to decreased muscle mass and increased adiposity within muscle.

INTRODUCTION

Intramuscular fat deposition within the musculature starts to become visible at the age of 12 months and the rate increases from 15 months to 24 months (Nishimura *et al.* 1999). The initial formation of visible intramuscular fat seems to be a result of the development of adipocytes (Pethick *et al.* 2006). Hocquette *et al.* (1998) reported that metabolic differences to balance triacylgriceride (TAG) storage within muscle such as fatty acid trafficking and oxidation of fatty acid in mitochondria are known as an important biological contributor in determining marbling levels in the later stages of finishing cattle.

Kokta *et al.* (2004) reviewed the interaction between myogenic cells and adipocytes to determine the rate and extent of myogenesis and adipogenesis during animal growth. Fat and muscle development are regulated by a number of complex biological pathways such as adenoreceptor signaling (Fruhbeck *et al*. 2001), cytokine signaling pathway (Shin *et al*. 2003) and a wide range of hormonal and transcriptional factors. Therefore, marbling differences might be expressed by metabolic differences resulting from a complex mechanism of communication among cells (Sorisky *et al*. 1999).

This study identified differentially expressed genes in *M. longissimus dorsi* of animals with divergent marbling phenotypes. We selected high and low marbling animals and then looked at between-group differential expression of genes using the bovine genome array (Affymetrix Inc, USA).

MATERIALS AND METHODS

Animals. From a group of 90 steers, the 5 highest and the 5 lowest were selected based on the marbling score. Carcass measurements and intramuscular fat percentage (IMF % as defined by AOAC, 1990) of the muscle sample were measured. Summary statistics for the animals and muscle samples used in this study are shown in Table 1.

Table 1. Summary statistics of tissue sample for gene expression analysis

Groups	Animal	*EBV	Age (Month)	Marbling score (1-7)	IMF (%)
Low	509	0.37	26	2++	7.11
	537	0.2	27	2++	6.02
	554	0.4	27	3	4.88
	670	0.31	28	3	7.36
	691	0.2	28	3	12.04
High	527	1.02	26	7++	24.35
	547	1.015	27	7++	32.49
	586	0.7	31	7++	16.56
	589	0.69	28	7++	26.24
	632	0.415	28	7++	18.81

Target preparation and high-density array hybridization. Double stranded cDNA was synthesized from 3 μg mRNA using a Genechip Expression 3'-Amplification One Cycle Synthesis kit (Affymetrix Inc. USA). After the cDNA was purified, Biotin-labeled cRNA was synthesized in vitro using the Gene chip Expression 3'-Amplification reagents in the IVT labeling kit (Affymetrix Inc.). A hybridization cocktail (200 μl) containing 15 μg fragmented cRNA was injected into the Genechip Bovine Gene expression Array (Affymetrix Inc). The array was placed in a 45 hybridization oven at 60 rpm for 16 hours. The array was scanned with a GeneChipScanner 3000 (Affymetrix Inc.).

Data pre-processing. Data quality control and background correction were carried out using the statistical computing language R (http://www.R-project.org). All slides were deemed to be within normal quality standards. Expression intensities on a log2 scale were obtained from the probe level data using the R *affy* package (Gautier *et al.* 2004) for MAS5.0 (Affymetrix 2002), RMA (Irizarry *et al.* 2003) and GCRMA (Wu *et al.* 2003) methods. In MAS5.0, each probe was adjusted using a weighted average. All arrays were scaled to the same mean value for normalization (200) and were summarized by an adjusted log2 scale average using 1-step Tukey biweight. For RMA, the background was corrected by convolution. The data were quantile normalized and summarized by median polish. GCRMA background correction used an affinity measure model based on probe sequences and mismatch probe intensities. The data were filtered to remove control probe (n=131) and probes detected as marginal (M) and absent (A) in all arrays using MAS5 presence calls.

Statistical analysis. Genes differentially expressed between the high- and low marbling groups were detected using a moderated t-test in *limma* (Smyth 2004).

RESULTS AND DISCUSSION

Differentially expressed genes. A total of 136 differentially expressed genes (DEGs) were detected in 3 data-processing methods; MAS5.0 (65 transcripts), RMA (37 transcripts) and GCRMA (34 transcripts) (Figure 1). Of 136 DEGs, 21 were shown to be significant in at least 2 of the summarization methods (Figure 1). Of the 21 differentially expressed genes listed in Table 2, 8

DEGs were identified as up-regulated in the high marbling group and the remaining 13 DEGs were down-regulated in the low marling group. Based on the gene identities and associated function, 2 up-regulated genes are involved in steroid biosynthesis (squalene epoxidase and cytochrome P450). Six down-regulated genes (Arginyl-tRNA synthetase, Ribosomal protein S6 kinase, Thimetoligopeptidase 1, Proteosome activator subunit 4, ATP binding protein and CDC-like protein) belong to functional classes involved in DNA replication, protein synthesis and cell division. However, 7 DEGs are yet unidentified hypothetical proteins (single EST clones). Of these 7 DEGs, three hypothetical proteins (LOC788205, LOC509649 and LOC777601) and 2 transcribed loci (Bt.19107.2.A1_at and Bt.19107.1.S1) were identified as differentially expressed. These probes can be considered candidate genes for biochemical indicators of IMF accretion.

Gene Ontology (GO) analysis. GO terms were annotated onto the GO database (http://www.geneontology.org). The GO biological process is assigned to 10 categories at level 3 (Figure 1.B). Gene Ontology (GO) analysis shows that the 21 DEGs were mainly involved in primary metabolic process and cellular metabolic process in the biological process category, and more specifically in oxidation reduction and regulation of the development process.

Table 2. Differentially expressed genes in *M. longissimus dorsi* of high and low marbled Hanwoo steers

Probe ID	Gene Names	Fold Change	Significance in MAS, RMA and GCRMA
Bt.5323.1.S1_at	SH3 domain YSC-like 1	0.818	* * *
Bt.15675.1.S1_at	ADAM metallopeptidase with thrombospondin type 1	0.953	* * *
Bt.21021.1.S1_at	TBC1 domain family, member 7	0.712	* * *
Bt.2933.1.S1_at	Hypothetical protein LOC788205	0.668	* * *
Bt.9767.1.S1_a_at	Squalene epoxidase	0.867	* * *
Bt.621.1.S1_at	Cytochrome P450, family 51, subfamily A	0.525	* * ns
Bt.23903.1.A1_at	Unknown	-0.53	* * ns
Bt.22362.1.S1_at	Similar to SH3-domain kinase binding protein 1	-0.96	* * ns
Bt.16752.1.A1_at	ATP binding protein	-0.693	ns * *
Bt.1020.1.S1_at	Similar to CDC-like kinase 1	-0.408	ns * *
Bt.19107.2.A1_at	Transcribed locus	-0.548	ns * *
Bt.28011.1.S1_at	Unknown	-1.066	ns * *
Bt.22718.1.A1_at	Proteasome (prosome, macropain) activator subunit 4	-0.326	ns * *
Bt.19107.1.S1_at	Transcribed locus	-0.642	ns * *
Bt.25102.1.S1_a_at	Hypothetical LOC509649	-0.496	ns * *
Bt.22038.1.S1_a_at	Similar to Arginyl-tRNAsynthetase	-0.215	ns * *
Bt.21268.1.S2_at	Ribosomal protein S6 kinase, 70kDa, polypeptide 1	0.459	ns * *
Bt.13342.1.S1_at	Similar to Src-associated protein SAW	-0.383	ns * *
Bt.344.1.S1_at	Major histocompatibility complex, class II, DM alpha	-0.595	ns * *
Bt.21827.2.S1_at	Thimetoligopeptidase 1	-0.818	* * ns
Bt.21794.1.S1_at	Hypothetical protein LOC777601	1.1243	* * ns

CONCLUSIONS

The analysis detected 2 genes which were upregulated in highly marbled cattle (Squalene epoxidase and Cytochrome P450) and thought to play a role in the fatty acid oxidation/reduction pathway. In addition there were 6 downregulated genes (Arginyl-tRNA synthetase, Ribosomal protein S6 kinase, Thimetoligopeptidase 1, Proteosome activator subunit 4, ATP binding protein

and CDC-like protein) which are involved in protein synthesis. This indicates that increased marbling in cattle was associated with down-regulation of genes involved in the cell division cycle and protein synthesis and upregulation of genes associated with adiposity within the muscle.

(A) **(B)**

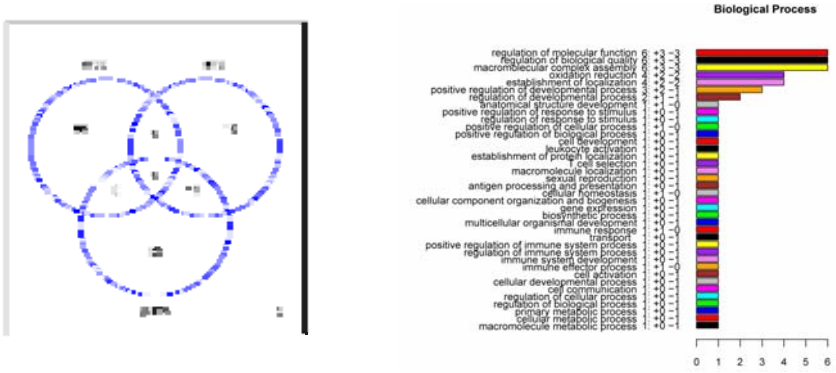

Figure 1. (A) Distribution of the 136 differentially expressed genes on intramuscular fat (IMF) in three data-processing methods (MAS5.0, RMA and GCRMA). (B) GO annotation (biological process term) for 21 differentially expressed genes.

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REFERENCES

Affymetrix (2002) Statistical algorithms description document

AOAC (1990) Association of Official Analytical Chemists, Washington, DC, USA

Fruhbeck, G., Gomez-Ambrosi, J and Burrell, M.A (2001) Am J Physiol. **280**:E827.

Gautier, L., Cope, L., Bolstad, B.M., Irizarry, R.A (2004) Bioinformatics **20**:307.

Hocquette, J.F., Ortigues-Marty, I, Pethick, D and Fernandez, X (1998) Livest Prod Sci. **56**:115.

Kokta, T.A, Dodson, M.V., Gertler, A and Hill, R.A (2004) Domes Anim Endocrinol **27**:303.

Irizarry, R.A., Bolstad, B.M., Collin, F., Cope, L.M., Speed, T.P (2003) Nucleic Acids Research **31:** e15.

Nishimura, T., A. Hattori and K. Takahashi (1999) J Anim Sci, **77**:93.

Pethick, D.W., Harper, G.S., Hocquette, J.F and Wang, Y.H (2006) Aust beef-the leader conference, 103.

R Development Core Team (2008). R: A language and environment for statistical computing.

Shin, S.M et al (2003) FEBS Lett. **543**:25.

Smyth, G.K (2004) Statistical Applications in Genetics and Molecular Biology **3:**3.

Sorisky, A (1999) Crit Rev Clin Lab Sci. **36**:1.

Zhijin, Wu et al (2003) Journal of the American Statistical Association **99:** 909.