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Identification of Functional and *In silico* Positional Differentially Expressed Genes in the Livers of High- and Low-marbled Hanwoo Steers

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ABSTRACT : This study identified hepatic differentially expressed genes (DEGs) affecting the marbling of muscle. Most dietary nutrients bypass the liver and produce plasma lipoproteins. These plasma lipoproteins transport free fatty acids to the target tissue, adipose tissue and muscle. We examined hepatic genes differentially expressed in a differential-display reverse transcription-polymerase chain reaction (ddRT-PCR) analysis comparing high- and low-marbled Hanwoo steers. Using 60 arbitrary primers, we found 13 candidate genes that were upregulated and five candidate genes that were downregulated in the livers of high-marbled Hanwoo steers compared to low-marbled individuals. A BLAST search for the 18 DEGs revealed that 14 were well characterized, while four were not annotated. We examined four DEGs: ATP synthase F0, complement component CD, insulin-like growth factor binding protein-3 (IGFBP3) and phosphatidylethanolamine binding protein (PEBP). Of these, only two genes (complement component CD and IGFBP3) were differentially expressed at $p<0.05$ between the livers of high- and low-marbled individuals. The mean mRNA levels of the PEBP and ATP synthase F0 genes did not differ significantly between the livers of high- and low-marbled individuals. Moreover, these DEGs showed very high inter-individual variation in expression. These informative DEGs were assigned to the bovine chromosome in a BLAST search of MS marker subsets and the bovine genome sequence. Genes related to energy metabolism (ATP synthase F0, ketohexokinase, electron-transfer flavoprotein-ubiquinone oxidoreductase and NADH hydrogenase) were assigned to BTA 1, 11, 17, and 22, respectively. Syntaxin, IGFBP3, decorin, the bax inhibitor gene and the PEBP gene were assigned to BTA 3, 4, 5, 5, and 17, respectively. In this study, the *in silico* physical maps provided information on the specific location of candidate genes associated with economic traits in cattle. (**Key Words :** Differentially Expressed Genes, High- and Low-marbled Liver, ddRT-PCR)

INTRODUCTION

Marbling (intramuscular fat) plays an important role in determining the juiciness and tenderness of beef and is one of the main factors used to grade beef quality in the United States (United States Department of Agriculture (USDA), 1989), Japan (Japanese Meat Grading Association (JMGA), 1988), and Korea. Therefore, marbling is a very important and valuable trait in the cattle industry. Muscle is the major target tissue for intramuscular fat development, and several reports have reported gene expression profiling using cDNA microarrays and differential-display reverse transcription-polymerase chain reaction (ddRT-PCR) methods to identify the genes regulating marbling within muscle tissue (Child et al., 2002; Wang et al., 2005). However, no study has examined hepatic gene expression profiles related to these traits. Most dietary long-chain fatty acids (LCFA) and nutrients bypass the liver and produce plasma lipoproteins, such as TG-rich very low-density lipoprotein (VLDL) and chylomicrons (Bauchart, 1993; Grum et al., 2002). These plasma lipoproteins transport lipids from the secreting organ (liver and intestine) to the target tissue (adipose tissue, muscle, and mammary glands). Therefore, it may be important to understand the metabolic mechanism of dietary fatty acids in the liver. Ponsuklili et al. (2005) found that single nucleotide polymorphisms (SNPs) of hepatic differentially expressed genes (DEGs) were associated with economically important traits in the pig, such as obesity and muscularity. Recent advances in high-throughput gene expression technology, such as cDNA microarrays and ddRT-PCR, have facilitated research to identify DEGs

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Table 1. Primer for quantitative real-time reverse transcription PCR

Gene	Primer sequence (5'→3')
Complement component CD	AAGGAGGGGGCACACTATCT
	TGCGTGGACAGTGTGTATGA
Phosphatidylethanolamine binding protein	CTGACCACTCAGGGCTTAGG
	ATTCAAGGTGGCCTCTTTCC
ATP synthase F0	TTGTTAGTAGGATTAGAATGGTGAATG
	CTGGAACACCCACTCCACTAA
Insulin-like growth factor binding protein 3	CTTGTTTCCAAGCAGTGCAG
	CCTCCACTTCATGCCTTAGC
RPLR gene (Internal control)	CAA CCC TGA AGT GCT TGA CAT
	AGG CAG ATG GAT CAG CCA

related to economic traits, such as marbling. Particularly, the entire bovine genome sequence has been published in GenBank (http://hgdownload.cse.ucsc.edu/goldenPath/bosTau2/chromosomes/), and it is possible to map the physical location of informative DEGs on the genome sequence. In this study, we identified 18 DEGs in the livers of high- and low-marbled Hanwoo cattle (Korean native cattle) using the ddRT-PCR method and mapped the physical location of these DEGs on the bovine genome sequence using an *in silico* BLAST search.

MATERIALS AND METHODS

Animals, diet and sampling

Ninety Hanwoo male were selected at 1 month old with the similar birth weight (24.8±3.8 kg) and castrated at 3 months old. All steers were fed the same diets in a feedlot house. The concentrates were offered at 1.5% and 1.8% of body weight on growing (-6 months) and early fattening stage (6-12 months), respectively, and in late fattening stage (13-27 months) steers were fed high concentrate grain diet *ad libitum*. Steers were fed forage *ad libitum* and had free access to fresh water during the whole period. The animals were humanely killed at 27 months old at the slaughter house of NIAS (National Institute of Animal Science). All Hanwoo tissue samples (liver, longissimus dorsi, rump and subcutaneous adipose tissue) were taken and immediately frozen in liquid nitrogen and stored at -80°C until analysis. Of ninety Hanwoo steers, we have taken each seven liver samples (three individuals for prescreening, and four individuals for validation) in high (marbling grading 6-7) and low (marbling grading 2-3) marbled Hanwoo steers (a marbling score of Beef Marbling Standard was assessed on a 1 to 7 scale, with 7 the highest (Kim et al., 2003)).

Total RNA extraction

The total RNA samples was prepared from liver for the ddRT-PCR prescreening using Trizol reagent (Life Technologies Inc., Grand Island, NY, USA) according to the manufacturer's instructions and quantified by absorbance at 260 nm.

ACP-based differential display RT-PCR

First-strand cDNA synthesis was performed for 1.5 h at 42°C in a final reaction volume of 20 μl containing purified total RNA 3 μg, 4 μl of 5×reaction buffer, 5 μl dNTPs (each 2.5 mM), 2 μl of 10 μm cDNA synthesis primer dT-ACP1, 0.5 μl of RNase Inhibitor (40 U/μl; Promega) and 1 μl superscript II reverse transcriptase (200 U/μl; Invitrogen, Carsbad, CA). The synthesized first-strand cDNA samples were diluted by the addition of 100 μl ultra-purified water. Polymerase chain reaction (PCR) amplification was conducted using the same GeneFishing™ DEG kits (Seegene, Seoul, Korea) in 50 μl reaction volume, consisting of 10×buffer without $MgCl_2$, 25 mM $MgCl_2$, 5 μm arbitrary ACPs, 10 μm dT-ACP2, 2 mM dNTP, 2.5 U Taq DNA polymerase (applied biosystems, USA) and 1 μl of first-strand cDNA, using a PTC 225 DNA engine (Biorad, USA). Each kit comprises 20 different arbitrary annealing control primers. The program of PCR amplification was 1 cycle at 94°C for 5 min, 50°C for 3 min and 72°C for 1 min, 40 cycles at 94°C for 40 second, 65°C for 40 second, 72°C for 40 second and 72°C for 5 min. The PCR products were separated by 2% agarose gel. The bands were photographed using Polaroid film under ultraviolet light after ethidium bromide staining and analyzed.

Cloning and sequence analysis

Differentially expressed bands were extracted and cloned into a TOPO TA cloning vector (Invitrogen, Karlsruhe, Germany). To confirm the identities of insert DNA, isolated plasmids were sequenced using a ABI3730XL sequencer (Applied Biosystems, USA). Complete sequences were analyzed by searching for similarities using a BLASTX program (Altschul et al., 1990) at the National Center for Biotechnology Information (NCBI).

Quantitative real-time PCR confirmation for selected genes

In this study, we identified 18 differentially expressed genes in each three samples of marbling high and low individuals. Of these genes, we selected 4 differentially

Table 2. Identification of differentially expressed gene between marbling high and low cattle liver

Clone	Gene name	Expression pattern[1]	Length (bp)[2]	Accession No	p-value
1	Unknown	Up	300	-	-
2	NADH dehydrogenase	Up	200	TC276581	5.7e-19
3	Ketohexokinase	Up	600	TC275323	1.8e-106
4	Syntaxin	Down	200	TC279167	0.93
5	Heat shock protein beta 1 (growth related protein)	Up	250	TC289298	6.2e-37
6	No sequencing	Up	250	-	-
*7	ATP synthase F0, subunit	Down	300	TC262494	7.7e-60
8	Bax inhibitor gene	Up	1,200	TC276625	1.3e-146
*9	Insulin-like growth factor binding protein-3	Up	900	TC262713	1.8e-134
10	Unknown	Up	200	TC279652	4.1e-20
11	Electron-transfer flavoprotein-ubiquinone Oxidoreductase	Up	1,200	TC278410	3.4e-155
12	My005 protein (Thymocyte protein)	Up	700	TC288220	3.0e-84
13	RNA cyclase	Down	300	TC266731	4.2e-40
*14	Complement component C3D	Up	400	TC274826	4.4e-59
15	Phosphatidylinositol 4-kinase	Down	270	TC276944	3.9e-63
16	Unknown	Up	900	TC260229	4.3e-155
17	Decorin precursor (Bone proteoglycan II)	Down	400	TC262564	1.5e-68
*18	Phosphatidylethanolamine-binding protein	Up	600	TC262839	2.9e-102

[1] That means is expression pattern between high and low marbled liver. Up is highly expressed in high marbled liver, Down is highly expressed in low marbled liver.
[2] The product size of differential display RT-PCR.
* These genes were confirmed by using quantitative realtime PCR.

expressed genes to validate ACP ddRT-PCR prescreening. The selected genes for ACP ddRT-PCR analysis validation were insulin like growth factor 3, phosphatidyl ethanolamine binding protein, ATP synthase F0 and complement component CD. Sequence-specific primers were designed to amplify products ranging from 150 to 300 bp using the program primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi; Table 1). First strand cDNA was synthesized from 3 μg total RNA from each four individual animal using the random hexamer primers (Promega Co., Madison, USA) and the superscript reverse transcriptase enzyme (Invitrogen Co., Grand Island, NY, USA). The mRNA expression levels for the selected genes were analysed by quantitative real-time PCR with specific primer (Table 1). 18S rRNA gene (GenBank accession No. AY779626) was used as an internal control. Realtime RT-PCR amplification mixtures (20 μl) contained 1 μl cDNA, 2×SYBR Green I Master Mix (10 μl) (Qiagen., GmbH, Germany), and 10 pM forward and reverse primers. The real-time PCR reactions started at 95°C for 15 min for predenaturation, and the condition was set at 95°C for 10 second, 58°C for 20 second and 72°C for 30 second. The PCR cycle performed 40 cycles. The PCR were conducted in ABI 7500 system (Applied biosystems, USA). The relative quantification of target genes expression were evaluated using the ΔC_T method. The ΔC_T value was determined by nomalizing the 18S ribosomal C_T value for each sample from the target C_T value. Fold change in the relative gene expression of target were determined by calculating the $2^{-\Delta CT}$.

In silico mapping of differentially expressed genes

The differentially expressed genes (DEG) and subset of MS marker from 1997 MARC map (Kappes et al., 1997) were compared by BLASTN to the draft assembly of the bovine whole-genome sequence using the NLRI (National Institute of Animal Science, RDA, Korea) interface (http://www.nabc.go.kr). An *E*-value of e^{-4} was considered significant for identification of bovine orthologues. Significant matches were returned for 16 of the 18 differentially expressed genes (89%). Distribution of these DEG sequences and nearest MS marker informations on the bovine genome sequence is presented in Table 2.

Statistical analysis

Results of realtime PCR analysis are given as means±SD. Statistical significance was determined using the Student's t-test. The level of significance used in all studies was $p<0.05$.

RESULTS AND DISCUSSION

DEGs in the livers of high- and low-marbled Hanwoo steers

The ddRT-PCR method has a high false positive rate. Recently, a new annealing control primer (ACP) ddRT-PCR method was developed. The ACP ddRT-PCR method is based on the unique tripartite structure of a specific primer, which has a 3'-end region with a target core nucleotide sequence, a 5'-end region with a nontarget universal nucleotide sequence, and a poly (dI) linker bridging the

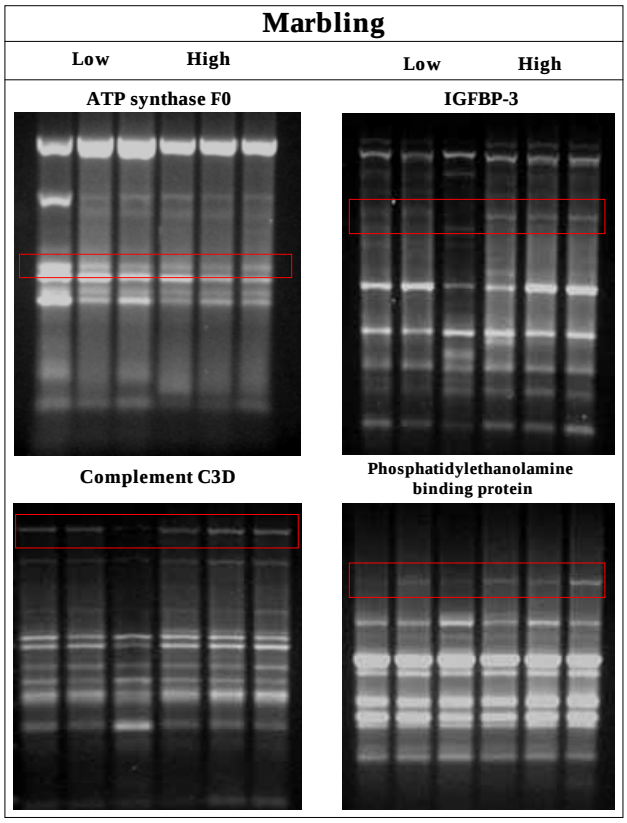

Figure 1. Gel image for the differential banding patterns obtained from Hanwoo high and low marbled liver samples. The ACP DDRT-PCR products are separated on 1.5% agarose gel and stained with ethidium bromide. The red box indicated differential expressed bands selected in high and low marbled Hanwoo liver.

3'- and 5'-end sequences (Hwang et al., 2003; Kim et al., 2004). These 5'-end nontarget and poly (dI) linker sequences do not interrupt nonspecific hybridization. Therefore, this method results in a highly reproducible PCR reaction. We used ACP ddRT-PCR to isolate DEGs from the livers of high- and low-marbled Hanwoo steers using a combination of 60 arbitrary primers and two anchored oligo (dT) primers from the ACP-based GeneFishing PCR kit (Seegene, Korea). Using the 60 arbitrary primers, we amplified about 500 bands. Of these 500 amplified bands, we found 18 differentially expressed bands, of which 13 were upregulated in high-marbled meat and five were downregulated. The 18 differentially expressed band fragments were extracted from gels and cloned into TOPO-TA cloning vector (invitrogen, Karlsruhe, Germany). These cloned band fragments were sequenced using 3730XL DNA sequencer (Applied Biosystems, USA) (Figure 1 and Table 2). The DNA sequences of these clones were analyzed using the BLAST search interface in the TIGR gene index, which showed that 14 of the differentially expressed clones were well characterized and four clones were not annotated in bovine gene pools. The 14 differentially expressed clones were classified using a GeneOntology search. Of these, NADH hydrogenase, ketohexokinase, ATP synthase F0, and electron-transfer flavoprotein-ubiquinone oxidoreductase are related to the mitochondrial respiratory chain. The major functions of mitochondria are to produce ATP energy and conduct oxidative phosphorylation and fatty acid β-oxidation. The mitochondrial metabolic cycle in human muscle and liver is related to disease and aging (Honokio et al., 1995; Nicoletti et al., 2005). Ketohexokinase or hepatic fructokinase catalyzes the first step in the metabolism of dietary fructose to produce energy, converting fructose into fructose-1-phosphate; malfunction of this enzyme causes diabetes mellitus (Hayward et al., 1996). Mitochondrial ATP synthase F0 plays a role in synthesizing ATP, which is the energy source derived from the proton gradient in mitochondria. The ATP synthase F0 subunit transports protons across the mitochondrial inner membrane to F1-ATPase (Fernando et al., 2005). Electron transfer flavoprotein exists in the mitochondrial matrix and has two heterodimer forms, involving 30 and 28 kDa subunits, and one flavin adenine dinucleotide (FAD) and one adenosine 5'-monophosphate (AMP). This enzyme is required for electron transfer from at least nine mitochondrial flavin-containing dehydrogenases to the main respiratory chain. Therefore, these mitochondrial respiratory genes are very important for generating ATP or FADH energy. Currently, in livestock genomics, these mitochondrial genes have not been associated with economically important traits. However, many genes derived from the mitochondrial genome were detected in our previous 23 K Affymetrix chip or ddRT-PCR analysis of the age- and fat-dependent phenotypes of Hanwoo cattle (data not shown). Therefore, it is necessary to study the function and structure of these DEGs derived from mitochondria further, to confirm their contribution to important livestock traits.

In addition, we identified phosphatidylinositol 4-kinase (PI4-K), which is involved in the phosphatidyl inositol cycle, which in turn is tied to cellular regulation by growth factor and hormones. The phylogenetic tree of the phosphoinositide superfamily contains three families: the phosphatidylinositol 4-kinase, phosphoinositol 3-kinase, and phosphatidylinositol related lipid/protein kinase families (Gehrmann et al., 1998). PI4-K (phosphoinositide-4-kinase) participates in mitogenesis, glucose transport, the regulation of hepatic glucose output, and insulin exocytosis in pancreatic beta cells (Gehrmann et al., 1998; Gromada et al., 2005). Recently, Kim et al. (2005) reported that SNPs within the PI3-K (phosphatidylinositol 3- kinase) gene were significantly associated with back and carcass fat in F_2 animals of a cross between Korean native and Landrace pigs. The phosphatidylethanolamine-binding protein (PEBP) is a raf kinase inhibitor protein (RKIP) that plays a role in the proliferation and differentiation of different cell

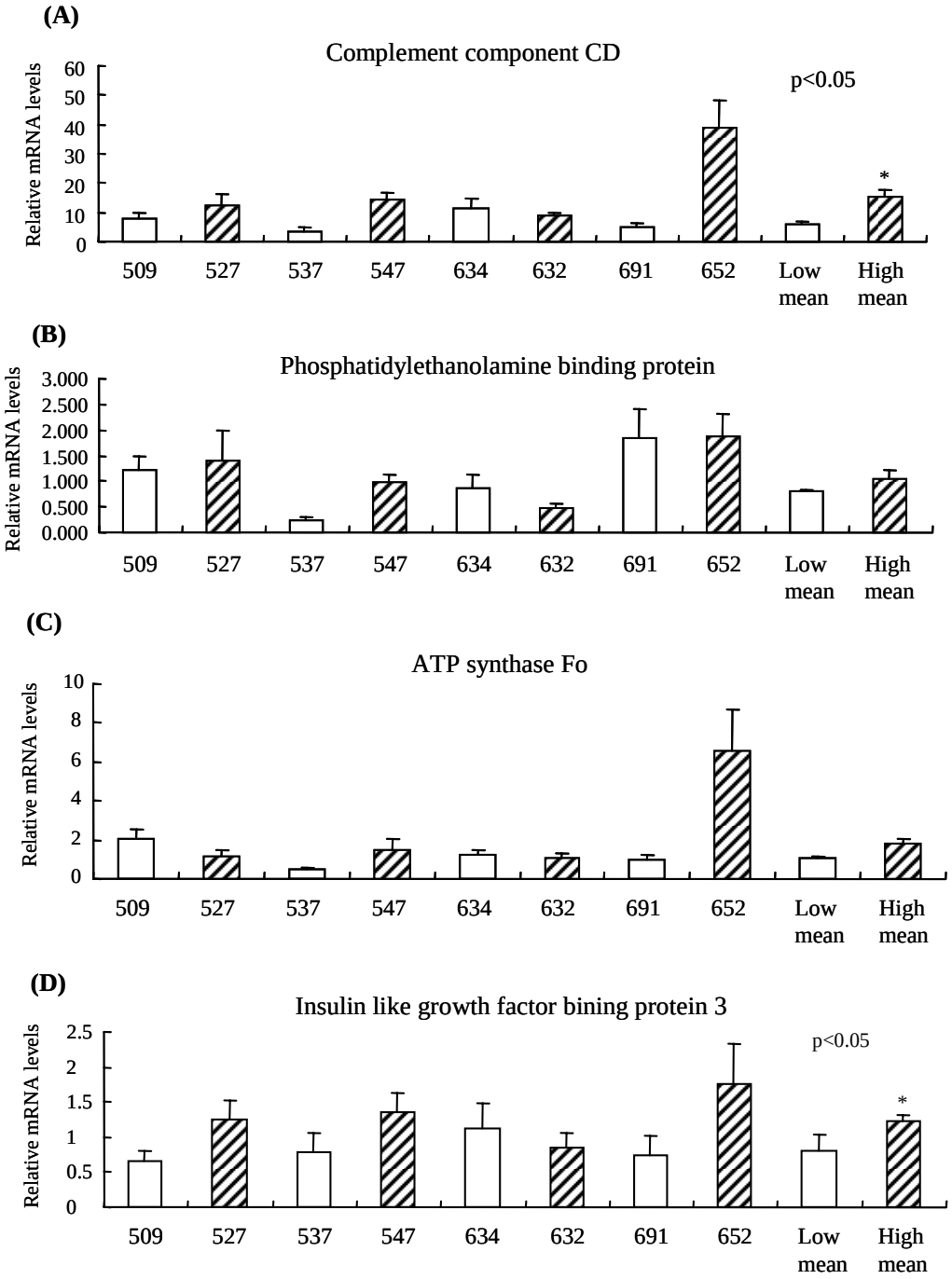

Figure 2. Relative transcript levels of four genes up-regulation in the high marbled animal liver obtained by quantitative realtime PCR analysis. Bars represent means of three repetitions, error indication show standard deviation. The open and striped bars represent low- and high-marbled Hanwoo steers's liver, respectively.

types (Yeung et al., 1999). RKIP suppresses the mitogen-activated protein kinase (MAPK) pathway. Recently, the RKIP gene was reported to play a role in cancer and Alzheimer's disease (Chen et al., 2006; Lee et al., 2006). The SNARE domain of syntaxin mediates a membrane fusion process, involving vesicular transport along the exocytic and endocytic pathways. Syntaxin 4 is the major plasma membrane SNARE for insulin-stimulated GLUT4 translocation in adipocytes. In addition, Min et al. (1999) found that syntaxin-binding protein 4 controls glucose transport and GLUT4 vesicle translocation. Therefore, the syntaxin gene may be related to fat deposition in cattle.

Quantitative real-time PCR confirmation of the selected genes

The transcripts of the selected genes were quantified in

Table 3. Summary of *in silico* physical mapping of bovine differentially expressed genes related intramuscular fat development

Gene ID	BTA No	Position	e-value	% ID	Match length (bp)	Nearest MS marker position	
						MS marker	Position
ATP synthase F0, subunit	BTA 1	72947550	3.10e-05	91.9	37	DIK5177	72901396
Syntaxin	BTA 3	27782032	1.70e-82	100	154	BM723	23407387
Insulin-like growth factor binding protein-3	BTA 4	43677725	0	96.6	560	ILSTS062	42112490
Decorin precursor (Bone proteoglycan II)	BTA 5	11421556	2.00e-141	99.6	257	DIK2138	11145444
Bax inhibitor gene	BTA 5	21630504	3.00e-104	84.5	375	BM321	20129042
Complement component C3D	BTA 7	12947807	1.00e-111	100	203	DIK2989	12539698
RNA cyclase	BTA 8	18454919	2.00e-109	100	199	BL1080	15419370
Ketohexokinase	BTA 11	53060904	4.00e-153	98.9	283	DIK2600	52955749
My005 protein (Thymocyte protein)	BTA 15	53467040	1.50e-41	83	176	DIK5195	53205446
Electron-transfer flavoprotein-ubiquinone oxidoreductase	BTA 17	21881168	5.80e-90	98.8	174	ILSTS023	18940318
Phosphatidylethanolamine-binding protein	BTA 17	32121818	0	99.6	474	DIK5227	31853688
Unknown	BTA 18	52047160	1.60e-53	99	109	BL1016	51209715
Unknown	BTA 19	24703066	0	97	682	IDVGA-46	23217624
NADH dehydrogenase	BTA 22	35955422	0.007	90.6	32	BMS693	34289141
Heat shock protein beta 1 (growth related protein)	BTA 25	31374053	1.10e-60	98.5	133	DIK4721	31174525
Unknown	BTA 26	25959723	8.00e-120	100	216	BMS2567	25910249

[1] Starting position is the nucleotide number on the bovine whole-genome assembly for the given chromosome.
[2] Percent ID is the sequence similarity determined from the aligned sequence match.
[3] Nearest MS marker position with the differentially expressed genes.

triplicate for eight individual samples. Of these four selected genes, only two genes (complement component CD and IGFBP3) were differentially expressed at $p<0.05$ between the livers high- and low-marbled Hanwoo steers. However, these genes are necessary to do further gene expression study using more many Hanwoo steers. Figure 2 shows the mRNA expression of these four genes, which were upregulated in high-marbled individuals. Particularly, the mean hepatic mRNA level of complement component CD in high-marbled individuals was twice as high as in low-marbled individuals, and the insulin-like growth factor binding protein 3 gene was 1.5 times higher than in low individuals. Complement component CD protein is a component of the complement activation pathway, which is part of the innate immune system that is capable of killing microorganisms directly and modulating phagocytosis, inflammation, and the humoral immune response (Wimmers et al., 2003). Generally, the immune system is closely connected to disease. Interestingly, there are several reports that the MHC haplotype influences growth traits in the mouse (Simpson et al., 1982), chicken (Briles, 1954), and pig (Jung et al., 1989). Immune system-related genes, such as MHC and complement genes, may be related to the disease or stress susceptibility of animals. Therefore, we need to identify SNPs of the complement component CD genes and examine their association with meat quality. The insulin-like growth factors, their receptors, and their binding proteins play key roles in regulating cell proliferation and apoptosis. Particularly, insulin-like growth factor (IGF) affects the proliferation and differentiation of muscle cells (Florini et al., 1996). IGFBP3 regulates IGF bioactivity to binding IGF (Jones and Clemmons, 1995). Johnson et al. (1999) reported that the IGFBP3 mRNA level is decreased significantly during differentiation of the myogenic cell line. Lee et al. (2005) reported that IGF-1 and IGFBP-3 have positive correlations with the live weight of cattle. In addition, Kim et al. (2005) reported 22 SNPs within IGFBP3 of Hanwoo cattle. Until now, no study has examined the relationship between IGFBP3 and meat quality. Therefore, we need to study the association between the SNPs of IGFBP3 and meat quality in Hanwoo cattle. In addition, although the mean mRNA levels of the PEBP gene and ATP synthase F0 gene were slightly higher in high-marbled individuals than in low-marbled individuals, the difference in the expression of these two genes was not significant.

In silico physical gene mapping

The differentially expressed genes (DEG) and subset of MS marker from 1997 MARC map (Kappes et al., 1997; Ihara et al., 2004) were compared by BLASTN to the draft assembly of the bovine whole-genome sequence using the NIAS (National Institute of Animal Science, RDA, Korea) interface (http://www.nabc.go.kr). An *E*-value of e^{-4} was considered significant for identification of bovine orthologues. Significant matches were returned for all of the 18 differentially expressed genes (100%). Distribution of these DEG sequences and nearest MS marker informations on the bovine genome sequence is presented in Table 3. Differentially expressed genes related to energy metabolism (ATP synthase F0, Ketohexokinase, Electron-transfer flavoprotein-ubiquinone oxidoreductase, and NADH

hydrogenase) were assigned to BTA 1, 11, 17, 22. The syntaxin, insulin-like growth factor binding protein 3, decorin, bax inhibitor gene, and phosphatidylethanolamine binding protein gene were assigned to BTA 3, 4, 5, 5 and 17, respectively. In this study the *in silico* physical maps provided information on the specific location of candidate genes associated with economic traits in cattle (Table 3).

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REFERENCES

Altschul, S. F., W. Gish, W. Miller, E. W. Myers and D. J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215(3):403-410.

Bauchart, D. 1993. Lipid apsorption and transport in ruminants. J. Dairy Sci. 76:3864-3881.

Briles, W. E. 1954. Evidence of overdominance of the B blood group alleles in the chicken. Genet. 39:961-965.

Chen, Q., S. Wang, S. N. Thompson, E. D. Hall and R. P. Guttmann. 2006. Identification and characterization of PEBP as a calpain substrate. J. Neurochem. 99:1133-1138.

Childs, K. D., D. W. Goad, M. F. Allan, D. Pomp, C. Krehbiel, R. D. Geisert, J. B. Morgan and J. R. Malayer. 2002. Differential expression of NAT1 translational repressor during development of bovine intramuscular adipocytes. Physiol. Genomics 10:49-56.

Fernando, M.-S., S. Wilkens and J. J. Garcia. 2005. Structure of dimeric mitochondrial ATP synthase: novel F0 bridging features and the structural basis of mitochondrial cristae biogenesis. Proc. Natl. Acad. Sci. USA. 102:12356-12358.

Florini, J. R., D. Z. Ewton and S. A. Coolican. 1996. Growth hormone and the insulin-like growth factor system in myogenesis. Endocr. Rev. 17:481-517.

Gehrmann, T. and L. M. G. Heilmeyer. 1998. Phosphatidylinositol 4-kinases. Eur. J. Biochem. 253:357-370.

Gromada, J. C., K. Bark, A. M. Smidt, J. Efanov, S. A. Janson, D. Mandic, W. Webb, B. Zhang, A. Meister, Jeromin and P-O. Berggren. 2005. Neuronal calcium sensor-1 potentiates glucose-dependent exocytosis in pancreatic b cells through activation of phosphatidylinositol 4-kinase beta. Proc. Natl. Acad. Sci. USA. 102:10303-10308.

Grum, D. E., J. K. Drackley and J. H. Clark. 2002. Fatty acid metabolism in liver of dairy cows fed supplemental fat and nicotinic acid during an entire lactation. J. Dairy Sci. 85:3026-3034.

Hayward, B. E., J. A. Fantes, J. P. Warner, S. Intody, J. P. Leek, A. F. Markham and D. T. Bonthron. 1996. Co-localization of the ketohexokinase and glucokinase regulator genes to a 500-kb region of chromosome 2p23. Mamm Genome. 7:454-458.

Hinokio, Y., S. Suzuki, K. Komatu, M. Ohtomo, M. Onoda, M. Matsumoto, S. Hirai, Y. Sato, H. Akai and K. Abe. 1995. A new mitochondrial DNA deletion associated with diabetic amyotrophy, diabetic myoatrophy and diabetic fatty liver. Muscle Nerve. 3:S142-149.

Hwang, In-Tak, Yun-Jee Kim, Seung Hyun Kim, Chae-il Kwak, Young-Yun Gu and Jong-Yoon Chun. 2003. Annealing control primer system for improving specificity of PCR amplification. BioTechniques 35:1180-1184.

JMGA. 1988. New beef carcass grading standards. Japan Meat Grading Association, Tokyo, Japan.

Jones, J. I. and D. R. Clemmons. 1995. Insulin-like growth factor binding protein: biological action. Endocr. Rev. 16(1):3-34.

Jung, Y. C., M. F. Rothchild, M. P. Flanagan, L. Christian and C. M. Warner. 1989. Association of restriction fragment length polymorphisms of swine leukocyte antigen class I genes with production traits of Duroc and Hampshire boars. Anim. Genet. 20(1):79-91.

Kappes, S. M., J. W. Keele, R. T. Stone, R. A. McGraw, T. S. Sonstegard, T. P. Smith, N. L. Lopez-Corrales and C. W. Beattie. 1997. A second-generation linkage map of the bovine genome. Genome. Res. 7(3):235-249.

Kim, C. J. and E. S. Lee. 2003. Effects of quality grade on the chemical, physical and sensory characteristics of Hanwoo (Korean native cattle) beef. Meat Sci. 63:397-405.

Kim, Yun-Jee, Chae-iI Kwak, Young-Yun Gu, In-Taek Hwang and Jong-Yoon Chun. 2004. Annealing control primer system for identification of differentially expressed genes on agarose gels. BioTechniques 36:424-434.

Kim, J. H., B. H Choi, H. T. Lim, E. W. Park, S. H. Lee, B. Y. Seo, I. C. Cho, J. G. Lee, S. J. Oh and J. T. Jeon. 2005. Characterization of phosphoinositide-3-kinase, class 3 (PIK3C3) gene and association tests with quantitative traits in pigs. Asian-Aust. J. Anim. Sci. 18:1701-1707.

Kim, J. Y., D. H. Yoon, B. L. Park, L. H. Kim, K. J. Na, J. G. Choi, C. Y. Cho, J. K. Lee, E. R. Chung, B. C. Sang, I. J. Cheong, S. J. Oh and H. D. Shin. 2005. Identification of novel SNPs in bovine insulin-like growth factor binding protein-3 (IGFBP3) gene. Asian-Aust. J. Anim. Sci. 18(1):3-7.

Lee, H. C., B. Tian, J. M. Sedivy, J. R. Wands and M. Kim. 2006. Loss of raf kinase inhibitor protein promotes cell proliferation and migration of human hepatoma cells. Gastroenterol. 131:1208-1217.

Lee, H. G., H. Hidari, S. K Kang, Z. S. Hong, C. X. Xu, S. H. Kim, K. S. Seo, D. H. Yoon and Y. J. Choi. 2005. The relationships between plasma insulin-like growth factor (IGF)-1 and IGF-binding proteins (IGFBPs) to growth pattern, and characteristics of plasma IGFBPs in steers. Asian-Aust. J. Anim. Sci. 18(11):1575-1581.

Min, J., S. Okada, M. Kanzaki, J. Elmendorf, K. Coker, B. Ceresa, L. Syu, Y. Noda, A. Saltiel and J. S. Pessin. 1999. A novel insulin-regulated syntaxin 4-binding protein mediating GLUT4 translocation in adipocytes. Molecular. Cell. 3:751-760.

Nicoletti, V. G., V. M. Marino, C. Cuppari, D. Licciardello, D. Patti, V. S. Purrello and A. M. Stella. 2005. Effect of antioxidant diets on mitochondrial gene expression in rat brain during aging. Neurochem Res. 30:737-752.

Ponsuksili, S., E. Murani, K. Schellander, M. Schwerin and K. Wimmers. 2005. Identification of functional candidate genes for body composition by expression analyses and evidencing impact by association analysis and mapping. Biochimica et

Biophysica Acta 1730:31-40.

Simpson, E., G. Bulfield, M. Brenan, W. Fitzpatrick, C. Hetherington and A. Blann. 1982. H-2 associated differences in replocated strains of mice divergently selected for body weight. Immunogenetics 15:63-70.

USDA. 1989. Official united states standards for grades of beef carcases. Agric. Marketing Serv. USDA, Washington, DC.

Wimmers, K., S. Mekchay, K. Schellander and S. Ponsuksili. 2003. Molecular characterization of the pig C3 gene and its association with complement activity. Immunogenetics. 54: 714-724.

Wang, Y. H., A. B. Keren, A. Reverter, G. S. Harper, M. Taniguchi, S. M. McWilliam, H. Mannen, K. Oyama and S. A. Lehnert. 2005. Transcriptional profiling of skeletal muscle tissue from two breeds of cattle. Mamm. Genome. 16:201-210.