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Effects of cell death-inducing DFF45-like effector B on meat quality traits in Berkshire pigs

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ABSTRACT. Cell death-inducing DFF45-like effector (CIDE) B is a member of the CIDE family of apoptosis-inducing factors. In the present study, we detected a single nucleotide polymorphism (SNP), *c.414G>A*, which corresponds to the synonymous SNP 414Arg, in *CIDE-B* in the Berkshire pigs. We also analyzed the relationships between the *CIDE-B* SNP and various meat quality traits. The SNP was significantly associated with post-mortem pH_{24h}, water-holding capacity (WHC), fat content, protein content, drip loss, post-mortem temperature at 12 h (T12) and 24 h (T24) in a co-dominant model ($P < 0.05$). A significant association was detected between the SNP and post-mortem pH_{24h}, fat content, protein content, drip loss, shear force, and T24 in gilts; and color parameter *b**, WHC, and T24 in barrows ($P < 0.05$). The SNP was significantly correlated with the fat content, and *CIDE-B* mRNA expression was significantly upregulated during

the early stage of adipogenesis, suggesting that *CIDE-B* may contribute towards initiation of adipogenesis ($P < 0.05$). Furthermore, *CIDE-B* mRNA was strongly expressed in the liver, kidney, large intestine, and small intestine, and weakly expressed in the stomach, lung, spleen, and white adipose tissue. These results indicate that the *CIDE-B* SNP is closely associated with meat quality traits and may be a useful DNA marker for improving pork quality.

Key words: *CIDE-B*; Meat quality trait; Berkshire pigs; Adipogenesis

INTRODUCTION

Pigs are one of the livestock, and pork quality is an economically important trait; thus, the pig breeding industry has focused on selecting pigs that produce high-quality pork. Pork quality has been evaluated by sensory attributes and biochemical parameters, such as intramuscular fat content, post-mortem pH, color, water-holding capacity (WHC), drip loss, tenderness, and cooking loss (Bonneau and Lebret, 2010).

The classical approach to improving meat quality is to select livestock according to their genealogy. Molecular markers and genomic maps have also been developed and applied gradually in the pig industry to determine the pork quality. A study reported that the single nucleotide polymorphism (SNP), *g.66686842 T>C*, in *DHPS* is closely associated with post-mortem pH (Zambonelli et al., 2013). Furthermore, four SNPs - *g.3247A>T*, *g.196A>G*, *g.392C>A*, and *g.955T>C* - in *GADD45A* were reported to have close associations with intramuscular fat content, drip loss, cooking loss, and WHC, respectively (Cho et al., 2015a).

Among the various pig breeds, black breeds typically have higher marbling scores, which is an indicator of good pork quality, than that of white breeds (Crawford et al., 2010). Particularly, Berkshire pigs have high-quality pork in terms of marbling, juiciness, tenderness, and flavor (Do et al., 2012; Lee et al., 2015). Thus, Berkshire pork is preferred in some countries, such as Korea and Japan (Crawford et al., 2010). Therefore, previously genetic markers, such as a SNP, *c.919G>A,* in *CBG* and two SNPs, *c.685T>G* and *c.776C>T*, in *EPHX1*, were identified to be associated with various pork quality traits (Cho et al., 2015b).

The cell death-inducing DNA fragmentation factor 45 (DFF45)-like effector (CIDE) is a family of apoptosis inducers, including CIDE-A, CIDE-B, and CIDE-C (Da et al., 2006). Proteins belonging to the CIDE family play important roles in regulating lipid metabolism, lipid droplet growth, and lipolysis (Xu et al., 2012; Singaravelu et al., 2013; Xu et al., 2016). *CIDE-B* is highly expressed in the liver, kidney, small intestine, colon, and white adipose tissue (WAT), but not in the brown adipose tissue and skeletal muscle, of mice (Li et al., 2007; Wu et al., 2008). In addition, *CIDE-B* mRNA expression is upregulated in the WAT, liver, lung, small intestine, lymphatic tissue, and brain of pigs (Li et al., 2016).

CIDE-B induces apoptotic cell death (Chen et al., 2000; Da et al., 2006). In addition, some studies suggest that apoptosis during post-mortem conditioning is associated with meat quality (Cao et al., 2010). Small heat shock proteins (sHSPs) are well known anti-apoptotic molecules that regulate meat quality (Ouali et al., 2006). Moreover, sHSPs are chaperones that maintain cellular homeostasis and ultimately affect meat quality (Lomiwes et al., 2014). Therefore, a study has suggested that apoptosis and caspase activities can affect post-mortem meat quality (Cao et al., 2010). Apoptosis often occurs under specialized cellular conditions,

such as cleavage of cytoskeletal proteins and cellular components, shrinkage of muscle cells, and loss of membrane asymmetry, which can lead to changes in meat quality (Sierra and Oliván, 2013). For example, caspase-3 and caspase-7 activities, which regulate apoptosis, have been measured in porcine skeletal muscle after slaughter. The semitendinosus muscle has significantly higher caspase-3 and caspase-7 activities than those in other muscles (Kemp et al., 2006). Furthermore, caspase-3 plays a role in early post-mortem meat quality (Huang et al., 2016).

CIDE-B also plays important roles in gluconeogenesis and glycogen synthesis. Lactic acid is generated by glycolysis in the muscles after slaughter (Scheffler and Gerrard, 2007). A strong relationship has been reported between glycogen content and post-mortem pH; pH increases when glycogen is degraded in the muscles during post-mortem glycolysis (Huang et al., 2014).

In this study, we hypothesized that *CIDE-B* might play an important role in the post-mortem pork quality of Berkshire pigs because of its role in apoptosis and gluconeogenesis. To test this hypothesis, we identified a SNP in *CIDE-B* and analyzed the correlation between pork quality and *CIDE-B* genotype. Moreover, we investigated the *CIDE-B* mRNA expression pattern among various porcine tissues as well as that during adipogenesis.

MATERIAL AND METHODS

Animals

Purebred Berkshire pigs were randomly selected and slaughtered when their body weight reached 110 kg. The pigs were reared under similar environmental conditions and were slaughtered by stunning with electrical tongs. The shocked pigs were exsanguinated while being hanged. The Animal Care and Use Committee of Gyeongnam National University of Science and Technology (GNTECH) specifically waived the need for consent because ethics committee approval is not required for slaughtering farm animals in the Republic of Korea. However, the pigs used in this study were slaughtered in accordance with the guidelines on animal care and use established by the Animal Care and Use Committee of GNTECH, and with the Korea Animal Protection Act and related laws.

Analysis of meat quality traits

Meat quality traits, such as backfat thickness, post-mortem pH, color, WHC, post-mortem temperature, Warner-Bratzler shear force, drip loss, and cooking loss, in addition to collagen, moisture, protein, fat, and ash contents, were evaluated as described previously (Cho et al., 2015b). Briefly, the longissimus dorsi muscle of Berkshire pigs (N = 416; 199 barrows and 217 gilts) was used in the experiments. Backfat thickness was measured at the tenth rib region, at a point of three-quarters along the muscle towards the belly. Post-mortem pH_{24h} was measured using a Hanna 9025 pH/ORP meter (Hanna Instruments, Woonsocket, RI, USA) with a penetration probe. Color parameters [CIE *L** (lightness), *a** (redness), *b** (yellowness)] were measured using a colorimeter (CR-400; Minolta, Tokyo, Japan). WHC was measured by centrifuging a muscle sample (0.5 ± 0.05 g) (Kristensen and Purslow, 2001). Post-mortem temperature (T) of the left half-carcass was measured after cooling in a conventional one-tier system for 24 h at 1°C. During carcass chilling and 12 and 24 h post-mortem, temperature

was measured using the aforementioned apparatus. Warner-Bratzler shear force was measured using the Illuminant C instrument (Hunter Association Laboratories, Inc., Reston, VA, USA). A 2-cm thick muscle slice (weight, 100 ± 5 g) was used to measure drip loss and a 3-cm thick slice (weight, 100 ± 5 g) was used to measure cooking loss after placing the samples in a polypropylene bag (Dongbang Co., Boryeong, Korea). The longissimus dorsi muscle (4 g) was added to 30 mL sulfuric acid solution in a conical flask to measure the collagen content. Moisture, protein, fat, and ash contents were analyzed according to the methods of the Association of Official Agricultural Chemists (AOAC, 2000) using the longissimus dorsi muscle samples obtained 24 h after slaughter.

RNA-sequencing

RNA-sequencing (RNA-seq) was performed by isolating total RNA from the liver tissues of Berkshire pig (N = 1) using TRI-Reagent (Molecular Research Center, Cincinnati, OH, USA), according to the manufacturer's instructions. The mRNA was purified using an RNA-Seq sample preparation kit (Illumina, Inc., San Diego, CA, USA). The mRNA was fragmented and used as a template for the first- and second-strand cDNA synthesis. The SNP was detected using a GAII analyzer (Illumina), as described previously (Jung et al., 2012).

Genotype analysis

Genomic DNA was isolated from the blood samples of 416 Berkshire pigs using the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA), according to the manufacturer's instructions. The SNP genotypes were analyzed using the Veracode Golden Gate assay kit (Illumina) to determine the association between the synonymous SNP and the pork quality traits. The oligonucleotides used in the genotype analysis are given in Table 1 and **Figure S1**.

Table 1. Summary for analysis of genotypes.

Locus		c.414G>A
Oligo-nucleotides for genotyping	Allele-frequency	G: 0.701 A: 0.299
	Allele-specific Oligo 1	5′-ACTTCGTCAGTAACGGACAGCTCCTCCGATGGGCCTCT-3'
	Allele-specific Oligo 2	5′-GAGTCGAGGTCATATCGTAGCTCCTCCGATGGGCCTCC-3'
	Locus-specific Oligo	5′-CACTGCTGCAAGGCCTGCTTCATCACGCCGGGTTAAAGCGTCTGCCTATAGTGAGTC-3'

Cell culture and pre-adipocyte differentiation

3T3-L1 murine pre-adipocytes were purchased from ATCC®CL-173™ (ATCC, Manassas, VA, USA) and maintained in Dulbecco's Modified Eagle's Medium (DMEM; Welgene, Daegu, Korea) supplemented with 10% bovine calf serum (BCS, Gibco, Grand Island, NY, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin (Welgene) at 37°C in a humidified atmosphere of air and 5% CO_2. The medium was changed every 2 days until the cells were 70% confluent. After they reached 70% confluence, 3T3-L1 cells were cultured in adipogenic differentiation medium containing DMEM supplemented with 10 µg/mL insulin, 0.5 mM 3-isobutyl-1-methylxanthine (IBM-X), and 1 µM dexamethasone (Sigma, St. Louis, MO, USA). Cells cultured for 1, 3, 5, and 7 days were used to evaluate *in vitro* adipogenesis.

Oil Red-O staining

Oil Red-O staining was performed to visually detect differentiation of adipocytes 7 days after induction, and to identify neutral lipids and fatty acids in the cells and tissues (Zhang et al., 2014). After differentiation, the 3T3-L1 cells were washed twice with phosphate-buffered saline, pH 7.4, and fixed in 3.7% (v/v) formaldehyde for 15 min. Then, the cells were incubated with 0.5% (v/v) Oil Red-O solution (6:4, prepared in isopropanol) for 30 min at room temperature. The excess Oil Red-O solution was washed off with distilled water and dried. The stained cells were visualized under a microscope.

Real-time quantitative polymerase chain reaction (qPCR) analysis

Total RNA was isolated from the liver, stomach, lung, kidney, large intestine, small intestine, spleen, and WAT of Berkshire pigs (N = 3) using TRI Reagent (Molecular Research Center). cDNA was generated using total RNA (2 µg), oligo (dT) primers, and the SuperScript™ II First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions, in a 20-µL volume.

Real-time qPCR was performed using a Rotor Gene-Q thermocycler (Qiagen, Valencia, CA, USA). In each reaction, 1 µL cDNA was used as the template and added to 10 µL reaction mixture (3 µL H_2O, 5 µL Rotor Gene SYBR Green PCR MasterMix (Qiagen), and 1 µL forward and reverse primers). The primer sequences for qPCR are listed in **Table S1**, and the reaction was performed for 40 cycles at 94°C for 5 s and at 60°C for 10 s. The peptidylprolyl isomerase A gene (*PPIA*) was used as an internal control. Amplification specificity was confirmed by performing melting curve analysis at 70°-95°C for 5 s. No-template controls were used as the negative control for qPCR analysis. A single sharp peak was detected for the target gene product.

The fold change in *CIDE-B* expression was analyzed by the $2^{-\Delta\Delta Cq}$ method after determining $^{\Delta\Delta}Cq$ yield as the relative difference between the experimental target gene and the reference gene, which was selected based on the tissue with the lowest *CIDE-B* mRNA expression level. All experiments were performed according to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments guidelines (Bustin et al., 2009). The Student *t*-test was used to detect differences.

Reverse transcription polymerase chain reaction (RT-PCR) analysis

Total RNA was isolated and cDNA was synthesized, using the method described above. RT-PCR was performed using gene-specific oligonucleotide primers. *CIDE-B* primer sequences and adipogenic marker genes are described in **Table S1**. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as an internal control. The *GAPDH* PCR was carried out for 20 cycles at 94°C for 30 s, 60°C for 20 s, and 72°C for 20 s. PCR for *CIDE-B* and adipogenic marker genes was carried out for 35 cycles at 94°C for 30 s, 60°C for 20 s, and 72°C for 20 s. The products were electrophoresed on 2% agarose gel in 6X loading buffer (Biosesang, Seongnam, Korea).

Statistical analysis

The general linear model procedure was used to detect differences between the

genotypes and meat quality traits using SAS software (ver. 9.1.3; SAS Institute, Cary, NC, USA). SNPs were selected for the statistical analysis according to call rate (>90%), minor allele frequency (MAF > 0.01), and Hardy-Weinberg equilibrium (HWE; P > 0.05).

The linear model was $y_{ijk} = \mu + G_i + S_j + P + e_{ijk}$, where y_{ijk} is the phenotypic value of the target trait, μ is the general mean, G_i is the fixed effect of genotype i, S_j is the fixed effect of gender j, P is the fixed effect of slaughter period, and e_{ijk} is the random error. A P value < 0.05 was considered significant.

RESULTS AND DISCUSSION

Analysis of the association between meat quality traits and genotype

Few studies have investigated the association between apoptosis and changes in meat quality (Ouali et al., 2006). In this study, SNPs from the liver, which is an essential metabolic organ and plays a key role in the metabolism of carbohydrates, proteins, and fats in the body, were analyzed (Mitra V, 2009). The *c.414G>A* SNP in *CIDE-B* was revealed as a change in the third Wobble base of 414Arg, resulting in a synonymous SNP (Table 1).

Next, the association between the *CIDE-B* genotype SNP and various pork quality traits was analyzed. The major allele was *G* (0.701) and the minor allele was *A* (0.299) (Table 1). The MAF (0.2969), HWE (0.1290), and call rate (95.19%) values were all suitable for the SNP analysis (**Table S2**).

In the dominant and co-dominant models, the *CIDE-B* genotype was significantly associated with post-mortem pH_{24h}, meat color CIE *L**, WHC, fat content, protein content, drip loss, T12, and T24. In the recessive model, *CIDE-B* genotype was significantly associated with backfat thickness, fat content, protein content, T12, and T24 (Table 2).

Table 2. Analysis of the association between pork quality traits and *CIDE-B* synonymous SNP *c.414G>A*.

Model	Dominant			Recessive		
Genotype	AA + AG	GG	P value	AA	GG + AG	P value
Backfat thickness (mm)	24.8203 ± 5.664	25.1910 ± 4.847	0.1664[ns]	22.8667 ± 6.377	25.1632 ± 5.164	0.0488*
Post-mortem pH_{24h}	5.7827 ± 0.194	5.8530 ± 0.222	0.0009***	5.7584 ± 0.117	5.8208 ± 0.215	0.2283[ns]
CIE *L**	48.9779 ± 2.935	48.3529 ± 2.809	0.0417*	48.8580 ± 2.575	48.6650 ± 2.915	0.8163[ns]
WHC (%)	57.9321 ± 2.712	58.4350 ± 2.585	0.0283*	57.5085 ± 3.402	58.2243 ± 2.593	0.0779[ns]
Fat (%)	2.6841 ±1.183	2.9115 ± 1.182	0.0093**	2.1378 ± 0.813	2.8438 ± 1.197	0.0006***
Protein (%)	1.5940 ± 0.727	1.6734 ± 0.742	0.007**	24.2117 ± 0.617	23.8183 ± 0.739	0.0018**
Drip loss (%)	4.786 ± 1.833	4.432 ± 2.042	0.0104*	4.9282 ± 1.457	4.5923 ± 1.974	0.1505[ns]
T12	17.4861 ± 3.164	16.8075 ± 3.222	0.0369*	18.4850 ± 3.394	17.0547 ± 3.182	0.0463*
T24	5.9886 ± 3.264	4.8703 ± 3.326	0.0016**	8.1350 ± 3.116	5.2452 ± 3.282	0.0001***
Model	Co-dominant					
Genotype	AA	AG	GG	P value	P value in AA *vs* AG	P value in AA *vs* GG	P value in AG *vs* GG
Post-mortem pH_{24h}	5.7584 ± 0.117	5.7866 ± 0.203	5.8530 ± 0.222	0.0039**	>0.05[ns]	>0.05[ns]	<0.01**
WHC (%)	57.5085 ± 3.402	58.0000 ± 2.589	58.4350 ± 2.585	0.0427*	>0.05[ns]	>0.05[ns]	>0.05[ns]
Fat (%)	2.1378 ± 0.813	2.7718 ± 1.211	2.9115 ± 1.182	0.0006***	>0.05*	>0.05***	>0.05[ns]
Protein (%)	24.2117 ± 0.617	23.8750 ± 0.734	23.7650 ± 0.742	0.0012**	>0.05*	>0.05**	>0.05[ns]
Drip loss (%)	4.9282 ± 1.457	4.7629 ± 1.889	4.4319 ± 2.042	0.0275*	>0.05[ns]	>0.05[ns]	>0.05[ns]
T12	18.4850 ± 3.394	17.3493 ± 3.119	16.8075 ± 3.222	0.0361*	>0.05[ns]	>0.05[ns]	>0.05[ns]
T24	8.1350 ± 3.116	5.6945 ± 3.182	4.8703 ± 3.326	0.0001***	>0.05**	>0.05***	>0.05[ns]

CIE *L** indicates meat color lightness. WHC indicates water-holding capacity. T12 and T24 represent the post-mortem temperature at 12 and 24 h, respectively. Significant differences between the genotype classes are indicated by *P < 0.05, **P < 0.01, and ***P < 0.001. [ns]Not significant difference.

In particular, the *CIDE-B* genotype was significantly associated with post-mortem pH_{24h} (P < 0.001) in the dominant model, and with fat content (P < 0.001) and T24 (P < 0.001) in the recessive and co-dominant models, respectively (Table 2). The *G* allele was associated with increase in post-mortem pH_{24h} and fat content and decrease in T12 and T24 (Table 2).

Accelerating the muscle chilling temperature improves pork quality due to a decreased rate of post-mortem metabolism (Jones et al., 1993; Kim et al., 2015; Rybarczyk et al., 2015). Therefore, the results of our study suggest that the *G* allele of *CIDE-B* affected pork quality traits to allow selection of high-quality pork.

In addition, the association between the *CIDE-B* genotype and the pork quality was analyzed according to sex. It was observed that the *CIDE-B* genotype was significantly associated with post-mortem pH_{24h}, fat content, protein content, drip loss, and shear force in gilts (Table 3).

Table 3. Association between pork quality traits in gilts and *CIDE-B* synonymous SNP *c.414G>A*.

Gender	Gilt						
Genotype	AA	AG	GG	P value	P value in AA *vs* AG	P value in AA *vs* GG	P value in AG *vs* GG
Post-mortem pH_{24h}	5.764 ± 0.026	5.769 ± 0.018	5.853 ± 0.019	0.0025**	<0.05[ns]	>0.05[ns]	<0.01**
Fat (%)	1.987 ± 0.197	2.175 ± 0.093	2.565 ± 0.093	0.0003**	>0.05[ns]	<0.01**	<0.01**
Protein (%)	24.347 ± 0.148	24.187 ± 0.065	23.912 ± 0.075	0.0019*	>0.05[ns]	>0.05*	>0.05*
Drip loss (%)	4.422 ± 0.271	4.589 ± 0.180	3.998 ± 0.164	0.0366*	>0.05[ns]	>0.05[ns]	>0.05*
Warner-Bratzler shear force (kg)	3.115 ± 0.144	3.092 ± 0.069	2.858 ± 0.066	0.012*	>0.05[ns]	>0.05[ns]	>0.05*

Significant differences between the genotype classes are indicated by *P < 0.05, **P < 0.01, and ***P < 0.001. [ns]Not significant difference.

However, the *CIDE-B* genotype was significantly associated with CIE *b**, WHC, and T24 in barrows (Table 4).

Table 4. Association between pork quality traits in barrows and *CIDE-B* synonymous SNP *c.414G>A*.

Gender	Barrow						
Genotype	AA	AG	GG	P value	P value in AA *vs* AG	P value in AA *vs* GG	P value in AG *vs* GG
CIE *b**	1.916 ± 0.301	2.744 ± 0.112	2.876 ± 0.101	0.014*	>0.05[ns]	>0.05*	>0.05[ns]
WHC (%)	55.887 ± 0.556	57.428 ± 0.253	58.107 ± 0.268	0.0133*	>0.05[ns]	>0.05*	>0.05[ns]
T24	10.500 ± 1.801	5.491 ± 0.415	4.301 ± 0.379	0.0019**	>0.05[ns]	<0.01**	>0.05[ns]

CIE *b** indicates meat color yellowness. WHC indicates water-holding capacity. T24 represents the post-mortem temperature 24 h. Significant differences between the genotype classes are indicated by *P < 0.05, **P < 0.01, and ***P < 0.001. [ns]Not significant difference.

Post-mortem pH_{24h} and fat content were significantly associated with the *CIDE-B* genotype in both the sexes. These results revealed that the *c.414G>A* SNP in *CIDE-B* had more effects on the meat quality in gilts than in barrows.

CIDE-B mRNA expression during adipogenesis

CIDE-B and the CIDE family share sequence homology and are highly expressed in the brown adipose tissue, which may play an important role in the development of obesity (Li et al., 2007). Furthermore, the important role of apoptosis induced by *CIDE-B* is mediated by regulation of lipid metabolism and storage (Xu et al., 2012). The porcine *CIDE-B* is located on chromosome 7 c.841 and is associated with fat deposition and metabolism (Li et al., 2016). In addition, *CIDE-B* can affect the variations in protein and shrinkage regulation of muscle during post-mortem meat conditioning (Rybarczyk et al., 2015). Therefore, a role for *CIDE-B* in fat regulation and pork meat quality could be anticipated.

Therefore, the *CIDE-B* mRNA expression level was evaluated to identify its role in adipogenesis. The 3T3-L1 cells were differentiated over 6 days. More than 90% of the cells

were mature adipocytes with numerous lipid droplets accumulated in the cytoplasm according to the Oil Red-O staining results (Figure 1A). *CIDE-B* mRNA expression was induced rapidly on days 1 and 3 of adipogenic differentiation (Figure 1B), suggesting a critical role for *CIDE-B* during the early stage of adipogenesis. The mRNA expression levels of the adipogenic marker genes - *ADIPOQ, LEP,* and *PPARG* - showed that the 3T3-L1 cells were well differentiated into adipocytes in the adipogenic culture medium (Figure 1C-E).
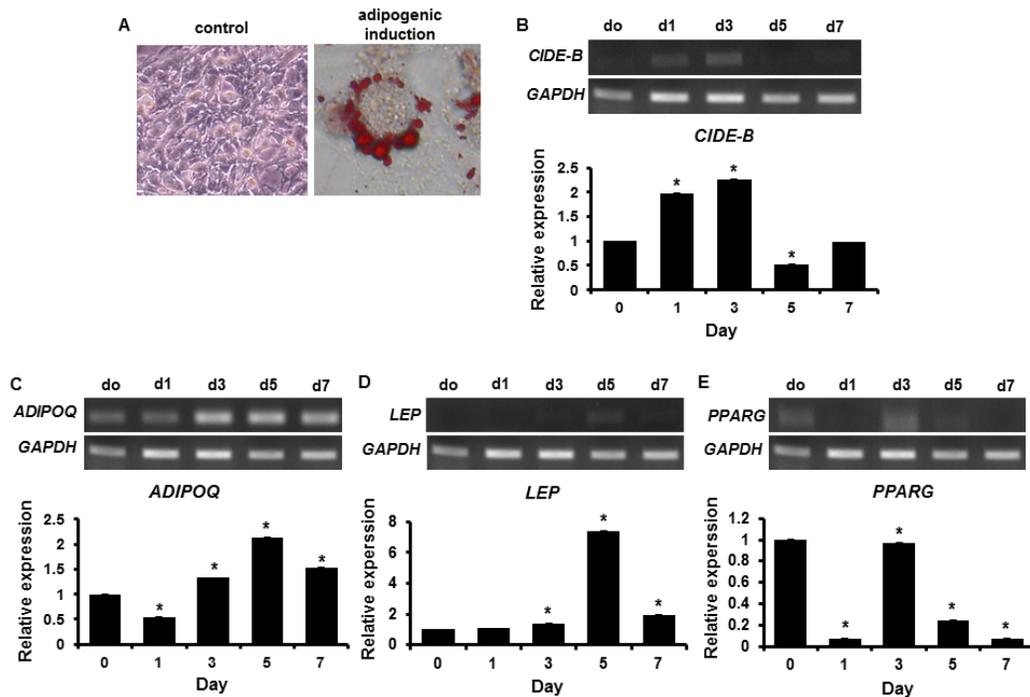

Figure 1. Cell death-inducing DFF45-like effector (*CIDE)-B* mRNA expression during adipogenesis. *CIDE-B* mRNA expression was measured by reverse transcription polymerase chain reaction (RT-PCR): 3T3-L1 pre-adipocytes were induced and differentiated with 10 μg/mL insulin, 0.5 mM IBMX, and 1 μM dexamethasone when they were 70% confluent. **A.** After 7 days of differentiation, the 3T3-L1 cells were stained with Oil Red-O. **B.** The fold-change in *CIDE-B* expression during adipogenesis was measured. **C.-E.** mRNA expression of adipogenic markers (*ADIPOQ*, *LEP*, and *PPARG*) by RT-PCR. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was the loading control. The statistical analysis was performed with Student's *t*-test. Data are represented as means ± SD. *P < 0.05 *vs* control.

Relative *CIDE-B* expression levels in various tissues of the Berkshire pig

Next, *CIDE-B* mRNA expression was evaluated in various pig tissues. A previous study reported that mouse *CIDE-B* mRNA is expressed significantly in the liver, kidneys, small intestine, colon, and WAT (Li et al., 2007; Wu et al., 2008). Another study reported that porcine *CIDE-B* is expressed in the WAT, liver, and small intestine (Li et al., 2016), and was expressed at significantly higher levels in the WAT of lean pigs than that in obese pigs (Li et al., 2016). It was predicted that *CIDE-B* would be expressed in the adipose tissue and might play

a crucial role in regulating the fat content of pork. Therefore, the *CIDE-B* expression in the adipose tissue and tissues that regulate fat content *in vivo*, such as the liver, was determined. *CIDE-B* mRNA expression was high in the liver, kidney, small intestine, and large intestine, but low in the lung, stomach, spleen, and WAT (Figure 2). The *CIDE-B* mRNA expression in WAT obtained in this study was different from that reported in the previous studies, which used fat pig breed. Because Berkshire is a lean pig breed, the result of the present study might be different from that of the previous studies owing to the strain differences. In addition, the *CIDE-B* mRNA expression level was low in WAT. The slaughtered pig was an adult, of which the adipose tissue was fully differentiated. The results of our study revealed that the *CIDE-B* mRNA expression was increased at the early stage of adipogenesis and decreased at the end of adipogenesis as shown in Figure 1. The low *CIDE-B* mRNA level in WAT might be due to the difference in preparation of WAT. In the future studies, *CIDE-B* mRNA expression in WAT of young pigs should be determined.

The liver is an important metabolic organ, which synthesizes glucose by glycogenolysis and plays a role in the conversion of muscle to meat via energy metabolism and acidification after slaughter (Henckel et al., 2002; Sterten et al., 2010). Therefore, *CIDE-B*, which was highly expressed in the liver, affects the glycogenolysis and adipogenesis, and can ultimately be associated to produce high-quality pork.
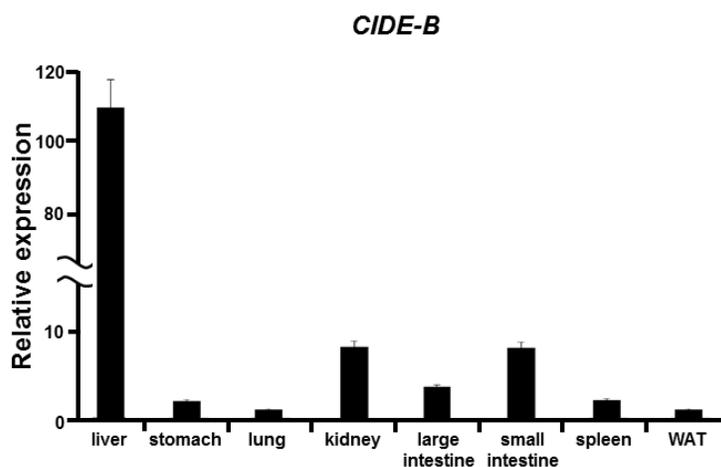

Figure 2. qPCR analysis of *CIDE-B* in various tissues of the Berkshire pig. Total RNA was isolated from each tissue, and cDNA was synthesized using oligo (dT) primers. *CIDE-B* mRNA expression level was examined by qPCR, normalized with a housekeeping gene (*PPIA*), and analyzed as relative-fold change compared with the lowest expression level of *CIDE-B* in the pig tissues. The statistical analysis was performed by Student's *t*-test. Data are represented as means ± SD. *P < 0.05 *vs* control.

In conclusion, we identified the *c.414G>A* SNP in *CIDE-B*, which was associated with various pork quality traits. *CIDE-B* mRNA expression increased significantly at the early stage of adipogenesis (days 1 and 3), suggesting that it might play a critical role in triggering adipogenesis. We also detected high *CIDE-B* mRNA expression levels in the liver of Berkshire pigs. These results suggest that the *CIDE-B c.414G>A* SNP could be an important molecular marker for improving pork quality.

To avoid variations, this study was performed using the pigs raised on the same farm, and can be regarded as those having genetic similarities; therefore, they can have an increased probability of containing similar SNP profiles. However, it was unlikely that there would be the possibility of familiar strains, since the farm, Dasan Pig Breeding Co., had a mating system with 15 boars. Nonetheless, further studies using Berkshire pigs raised on other farms are required.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary material

Figure S1. Position of oligonucleotides for genotype analysis. *CIDE-B* genotype analysis was performed using Veracode Golden Gate assay kit (Illumina). Chromosome start, end, and SNP position of *CIDE-B* are indicated. Genotype was analyzed using three oligonucleotides - Allele-specific Oligo 1 (ASO1), Allele-specific Oligo 2 (ASO2), and Locus-specific Oligo (LSO) - of chromosome positions.

Table S1. Primers for real-time qPCR and RT-PCR analyses.

Table S2. Summary of the CIDE-B SNP *c.414G>A* analysis.

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