Relationships among genome DNA methylation patterns in each upstream CG region on the 22 of genes controlled by epigenetic system and economic traits in Japanese Black cattle

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ABSTRACT: Heritabilities of quality, tenderness and marbling of meat of Japanese Black cattle (JB) are typically between 0.3 and 0.6, and meat of JB known as Wagyu has become very popular worldwide for its quality. However the main mechanism of developing their characteristic features are not well understood although many researchers have them reported SNPs and QTLs which may be concerning with them. DNA methylation to C in CG rich of the upstream region of start codon controls the expression of many genes on a genome wide level. This study's aims to examine relationships among DNA methylation pattern (DMP) and economic carcass traits in JB. DMP of genome DNA extracted from adipose tissues around the kidneys were analyzed with using specific primers for analyzing each upstream region of the 22 genes controlled by epigenetic system and some TAKARA's reagent kits. Correlations among some traits and DMPs were highly significant, and specific methylation patterns to C in some CG rich regions may have relation with rib loin thickness and carcass weight.

Keywords: Japanese Black cattle, economic traits, DNA methylation, upstream region

Introduction

Heritabilities of quality, tenderness and marbling of meat of Japanese Black cattle (JB) are typically between 0.3 and 0.6, and meat of JB known as Wagyu has become very popular worldwide for its quality. However the main mechanism of developing their characteristic features are not well understood although many researchers have them reported SNPs and QTLs which may be concerning with them. And moreover, major genes contributed to meat qualities among quantitative traits have not been reported, and also detection of candidate genes in QTLs is not enough because the analysis depending on sequencing polymorphism does not consider enough environmental effects according to growth and the detection performance to find out candidate genes is low. Today, we know that DNA methylation to CG rich regions in the upstream region of a start codon, ATG, controls the expression of many genes on a genome wide level, and a part of methylation patterns (DMP) is specific by each tissue and differentiation stage. In its process, it might reflect the environmental effects such as feeding condition, stress and so on. So the relationships among quantitative traits are affected by environmental effects and these might also be affected by DNA methylation. It is, therefore, important to include DMP should be included as a significant effect to evaluate accurately bovine's performances accurately. Kaneda et al. (2011) reported that the DNA methylation levels in the repetitive elements changed during bovine development, especially genome-wide reprogramming in germ cells. They selected 3 of important peri- or centromeric regions in chromosomes, Satellite I, II and art2 as repetitive sequences. These nuclear elements are expected to be on a genome wide level and they would affect efficiency of genome-wide gene expressions and improvement of productive performance as well (Li. (2002); Reik et al. (2007); Miyoshi et al. (2006); Jones et al. (2007); Hajkova et al. (2002); Lane et al. (2003)). This study's aims to examine relationships among DMP at the slaughter after fattening and economic carcass traits of JB siblings of two sires.

Materials and Methods

Genome DNA extraction procedure. Samples for genome DNA analysis were collected from adipose tissues around the kidneys in each 100 JBs produced from 2 sires (Sire A and B) have parent-child relation, totaling of 200 JBs. DNA midi Extraction Kit of TAKARA was used to extract and purify DNA samples to extract and purify DNA samples. EpiXplore Methylated DNA Enrichment Kit and a specific magnet stand of TAKARA were then used to concentrate and separate hyper-methylated DNA from DNA mixtures. To increase the detective sensitivity, arranged genome DNA samples were treated with a sonicator.

Amplification and evaluation of hyper-methylated DNA. To amplify a CG rich region in the promoter region, upstream region of start codon, ATG, EpiScope Promoter qPCR Array (Human) of TAKARA was used, and to detect and determine amplified volume, SYBR Premix Ex Taq GC (Perfect Real Time) of TAKARA was used. Prior to these analyses were carried out, we examined the sequence homology between human and bovine to make primer sequences specific to CG regions and confirmed the availability of all primers contained in the kit.

Bisulfate sequencing analysis. To perform the bisulfate sequence analysis of the amplified CG rich regions, TaKaRa Taq Hot Start Version and ABI's Big Dye Cycle Sequencing Kit, version 3.0 were used with ABI's 3130 Genetic Analyzer.

Data calculation and statistical analyses. To evaluate hyper-methylated DNA level, bound and unbound fractions in a tissue, a specific application suggested by TAKARA was used, and the graphs instructed by TAKARA were made accordingly. Correlation analysis and analysis of variance (ANOVA) are performed by using SAS program, version 9.1 in accordance with its operational manual.

Results and Discussion. The CG rich regions in the upstream regions in two genes, CAMD1 and RARB of

22 genes were low in DNA methylation level, and their mRNA expressions were considered to be activated. In the cattle produced from sire A, correlations between loin eye area (LEA) and ESR1, and bovine marbling score (BMS) and MLH1 were -0.45 and -0.48, respectively, at 5% significance level. In the cattle produced from sire B, correlations between rib loin thickness (RLT) and DKK3, carcass weight (CW) and ESR1, BMS and WT1 and BMS and DAPK1 were 0.57, -0.48, 0.41, -0.44 and -0.54 respectively, at 5% significance level. Specific methylation patterns related to some economic traits were found in the CG rich regions of APC, CDKN2A and CHFR. Apparent relationships between the known QTLs and these DMPs could not be found.

Conclusion

Significant correlations were found among some CG rich regions and economic traits. Specific methylation patterns related to some economic traits were also found in the three of the CG rich regions. However, the known QTLs by sequencing polymorphism did not relate with the DMP. These results show limitations of QTL and SNP analyses as methods to search for main genes contributing to quantitative traits of bovine and also importance of incorporating epigenetic information wich reflects einvironmental effectd into the analyses.

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Table 1. Correlations among carcass traits and methylation degree of 24 upstream regions in 22 genes of the cattle produced from sire A.

Gene	BMS	CW	LEA	RLT
APC(1)	-0.03	0.46	0.34	0.45
APC(2)	-0.54	-0.10	-0.22	-0.40
BNIP3	0.41	-0.08	-0.03	0.21
BRCA1	0.06	-0.10	-0.31	-0.02
CADM1	-0.37	-0.16	-0.28	-0.06
CCND2	-0.40	-0.01	0.42	-0.07
CDKN2A	0.03	-0.42	-0.34	-0.19
CDKN2B	0.05	0.13	0.16	0.33
CHFR	-0.09	-0.01	0.44	0.66
DAPK1	-0.11	-0.09	0.24	0.18
DKK3	0.38	-0.17	-0.02	0.02
ESR1	0.00	-0.36	-0.45	-0.29
FHIT	0.00	0.14	0.03	-0.07
LOX	-0.38	-0.03	-0.25	-0.03
MLH1	-0.48	-0.24	-0.21	-0.29
PTGS2	0.24	0.09	0.46	0.08
RARB	-0.15	-0.02	-0.26	0.02
RASSF1	-0.02	-0.05	-0.26	-0.04
RB1	0.18	-0.05	-0.23	0.22
VHL	0.12	-0.27	-0.21	-0.24
WT1	0.12	0.06	0.05	0.27
LINE-1	-0.40	0.11	-0.13	-0.01

BMS; bovine marbling score, CW; carcass weight, LEA; loin eye area, RLT; Rib loin thickness

Table 2. Correlations among carcass traits and methylation degree of 24 upstream regions in 22 genes of the cattle produced from sire B.

Gene	BMS	CW	LEA	RLT
APC(1)	0.26	0.39	0.28	0.24
APC(2)	0.04	0.14	0.45	0.13
BNIP3	0.26	-0.30	0.24	-0.1
BRCA1	0.24	-0.22	0.21	0.02
CADM1	-0.44	0.03	0.19	-0.22
CCNA1(1)	-0.23	0.49	-0.25	0.37
CCNA1(2)	-0.23	0.26	-0.15	0.54
CCND2	0.00	0.19	-0.04	0.26
CDKN2A	0.52	0.38	0.53	0.43
CDKN2B	0.2	-0.03	0.02	0.18
CHFR	-0.32	0.39	0.11	0.64
DAPK1	-0.54	-0.08	-0.32	-0.19
DKK3	0.02	0.29	0.13	0.57
ESR1	0.01	-0.48	0.09	0.1
FHIT	-0.01	0.00	0.30	0.28
GSTP1	0.37	-0.75	-0.69	-0.64
LOX	-0.28	0.03	-0.05	0.14
MLH1	0.11	0.28	0.25	0.07
PTGS2	-0.31	0.32	-0.02	-0.11
RARB	0.03	0.42	0.32	0.23
RASSF1	0.14	0.31	0.41	0.31
RB1	-0.10	-0.24	-0.27	-0.16
WT1	-0.44	-0.27	-0.23	-0.35
LINE-1	-0.01	0.20	-0.15	0.17

BMS; bovine marbling score, CW; carcass weight, LEA; loin eye area, RLT; Rib loin thickness