Mol Biol Rep (2013) 40:3231–3237
DOI 10.1007/s11033-012-2398-x

The SNP in the promoter region of the bovine *ELOVL5* gene influences economic traits including subcutaneous fat thickness

Hirokazu Matsumoto · Yumiko Shimizu · Atsuko Tanaka · Takuya Nogi · Ichiro Tabuchi · Kenji Oyama · Masaaki Taniguchi · Hideyuki Mannen · Shinji Sasazaki

Received: 2 August 2012 / Accepted: 17 December 2012 / Published online: 27 December 2012

Abstract Genetic analyses have contributed to improvements of economically important traits derived from adipose tissue such as fatty acid composition in beef. *Elongation of very long chain fatty acids* (*ELOVL*) genes encode for the enzymes that play an important role in elongation of long-chain fatty acids. In this study, we aimed to discover genetic polymorphisms of *ELOVL* gene family in cattle populations to develop genetic markers. As a result, five synonymous mutations were detected in the coding regions of the *ELOVL1, ELOVL2, ELOVL3* and *ELOVL5* genes. In addition, six mutations were identified in promoter region of the *ELOVL5*. Two of five mutations in the promoter region of *ELOVL5* were expected to alter the *ELOVL5* expression and influence the economic traits, because of the high synteny of the region which was essential for activation of *Elovl5* in mouse. Therefore, we performed association analysis between the genotypes and traits and our result revealed that T allele of g.-110T>C in *ELOVL5* gene promoter indicated significantly thinner subcutaneous fat thickness (TT, 2.39 cm; CT, 2.35 cm) than that of C allele (CC, 2.68 cm) in a Japanese Black population. Our results suggest that the g.-110T>C is a useful genetic marker for the breeding in beef cattle.

Keywords ELOVL · Subcutaneous fat thickness · SNP · Promoter · Japanese Black

Introduction

The Japanese Black cattle are the main source of domestic beef in Japan. A number of reports revealed some genetic factors to influence economic traits in beef cattle [1]. A nonsynonymous mutation in the *stearoyl-CoA desaturase* gene was associated with monounsaturated fatty acids (MUFA) percentage and melting point of intramuscular fat in Japanese Black cattle [2]. A single nucleotide polymorphism (SNP) in the *fatty acid binding protein 4* gene affected beef marbling score (BMS) and subcutaneous fat thickness in Japanese Black × Limousin F_2 crosses [3].

Elongation of very long chain fatty acids (*ELOVL*) genes encode the enzymes that play a major role in elongation of long-chain fatty acids. Fatty acid synthesis involves a number of enzymes such as fatty acid synthase (FASN) [4]. Mammalian FASN synthesizes fatty acids consisting of up to 16 carbons in length, and the fatty acids produced by the FASN are further elongated into long chain fatty acids containing 18 carbon atoms or longer by the ELOVLs, as well as fatty acids obtained from the diet [5]. The ELOVL enzymes are also known to be responsible for the rate-controlling of fatty acid elongation [6].

Electronic supplementary material The online version of this article (doi:10.1007/s11033-012-2398-x) contains supplementary material, which is available to authorized users.

H. Matsumoto · Y. Shimizu · A. Tanaka · H. Mannen · S. Sasazaki (✉)
Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe 657-8501, Japan
e-mail: sasazaki@kobe-u.ac.jp

T. Nogi · I. Tabuchi
Tottori Prefectural Agriculture and Forest Research Institute Livestock Research Center, Kotoura, Tottori 689-2503, Japan

K. Oyama
Food Resources Education and Research Center, Graduate School of Agricultural Science, Kobe University, Kasai, Hyogo 675-2103, Japan

M. Taniguchi
Animal Genome Research Unit, National Institute of Agrobiological Sciences, Tsukuba 305-8602, Japan

In mammals, seven enzymes have been identified as the ELOVL family (ELOVL1–7). Five or six transmembrane regions and a highly conserved HXXHH motif were commonly observed in all the members [7]. Each ELOVL enzyme has a distinct tissue distribution and individual enzymes exhibit different fatty acid substrate preferences [5, 8]. The ELOVL5 and ELOVL6 are involved in the production of palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0) and oleic acid (C18:1). Since they are major fatty acids in beef [9], the function of the ELOVL5 and ELOVL6 in fatty acid synthesis may be especially important for beef breeding.

The aim of this study is to discover additional genetic markers associated with carcass traits in beef cattle. For this purpose, we sought to identify DNA polymorphisms in the coding and promoter regions of *ELOVL* genes between cattle breeds. Statistical analysis was performed to investigate associations between polymorphisms and carcass traits.

Materials and methods

Animals and Traits

Four Japanese Black and four Holstein cattle were tested for sequence comparison in order to identify DNA polymorphisms. The Japanese Black cattle were sampled from diverse areas in Japan to represent the breed and the Holstein cattle from Tottori Prefecture. These cattle were chosen considering ancestry to minimize their genetic relationship. In order to sequence the coding region in the *ELOVL6* gene, cDNAs were prepared from subcutaneous adipose tissue. For sequencing the other *ELOVL* genes, genomic DNA samples were extracted from the *musculus trapezius*. For association study between the *ELOVL5* genotype and economic traits, three groups of animals were prepared. Genomic DNA were extracted from 556 Japanese Black cattle in Miyazaki Prefecture (JB1: 502 steers and 54 heifers), 225 Japanese Black cattle from diverse areas in Japan (JB2: 135 steers and 90 heifers) and 201 Holstein steers in Tottori Prefecture (HOL). The average ages in months ± SD of these groups at slaughter were 29.10 ± 1.62 (JB1), 28.54 ± 3.51 (JB2) and 20.50 ± 0.59 (HOL), respectively.

Carcass traits including dressed carcass weight (kg), rib-eye area (cm^2), rib thickness (cm), subcutaneous fat thickness (cm), yield estimate and BMS were measured by official graders of the Japan Meat Grading Association. In addition, fatty acid composition in beef was focused on in this study. For lipid extraction, the methods of Folch et al. and Morris et al. [10, 11] were applied. The perinephric fat tissue (JB1), the intramuscular fat of the *musculus longissimus thoracis* (JB2) and the intramuscular fat of the *diaphragm* (HOL) were sampled. Approximately 100 mg of fat was collected from each cattle; total lipids were extracted from these samples with chloroform:methanol (2:1, vol/vol). The lipids were methylated by the method of O'Keefe et al. [12] with sodium methylate. Methyl esters were analyzed with a gas chromatograph-equipped flame ionization detector (Hitachi G-3000, Tokyo, Japan). Analyzed fatty acid methyl esters were myristic acid (C14:0), myristoleic acid (C14:1), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2). The composition of each fatty acid was expressed as a percentage. MUFA, saturated fatty acids (SFA) and Elongation Index were calculated. MUFA included C14:1, C16:1 and C18:1 and SFA included C14:0, C16:0 and C18:0. Elongation Index indicated (C18:0+C18:1+C18:2)/(C16:0+C16:1).

Sequencing

Genomic DNA samples were used to sequence the coding sequence (CDS) regions of *ELOVL* genes except for *ELOVL6*. cDNA samples were used for the *ELOVL6*. The primers were designed based on GenBank sequences (*ELOVL1*: NC_007301, *ELOVL2*: NC_007324, *ELOVL3*: NC_007327, *ELOVL4*: NC_007307, *ELOVL5*: NC_007324, *ELOVL6*: NM_001102155 and *ELOVL7*: NC_007318). The promoter regions of the *ELOVL5* and the *ELOVL6* were also sequenced using genomic DNA samples. We focused on the regions homologous with the promoter regions of mouse *ELOVL5* and *ELOVL6* [13, 14]. The primers were designed based on GenBank sequences (*ELOVL5*: NC_007324 and *ELOVL6*: NC_007304). PCR was performed as the following: 35 cycles at 94 °C for 30 s, annealing temperature for 30 s, 72 °C for 1 min. The primer sequences and the annealing temperatures are summarized in Supplemental Table 1. For PCR, TaKaRa Ex Taq® Hot Start Version (Takara, Tokyo, Japan) or TaKaRa LA Taq® (TaKaRa) was used as polymerase. Sequencing was performed with BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). The sequence comparison was performed by DNASIS® Pro v3.0 (Hitachi, Tokyo, Japan). The sequence of the promoter regions in mouse *ELOVL5* and *ELOVL6* were referred to by GenBank sequences (*ELOVL5*: NC_000075 and *ELOVL6*: NC_000069). Putative transcription factor binding sites in the promoter region were searched by TFSEARCH ver 1.3 [15]. The locus of each SNP is identified by the distance in base pairs from the translation initiation site.

Phyloanalysis

For phyloanalysis among *ELOVL* family of human, cattle and mouse, a phylogenetic tree was constructed with MEGA4.0

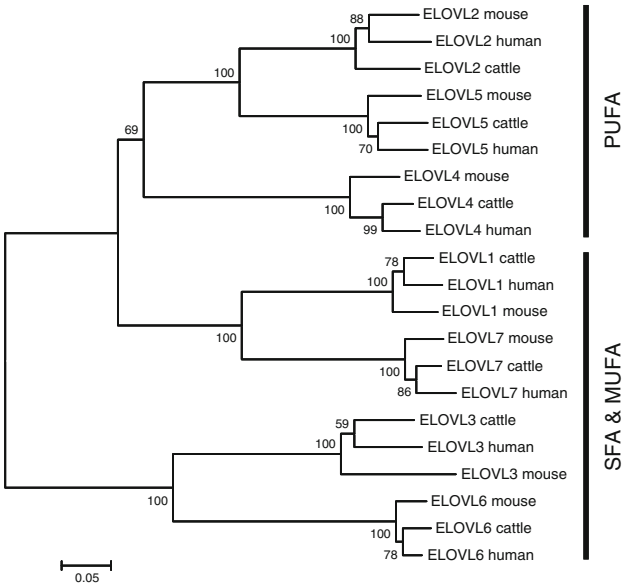

Fig. 1 The phylogenic tree of the *ELOVL* family. *ELOVL* family was divided into three groups: *ELOVL2*, *5*, *4*, *ELOVL1*, *7* and *ELOVL3*, *6*. The substrates of ELOVL2, 5, 4 were polyunsaturated fatty acids (PUFA), while those of the others were saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) [5, 8]. Bootstrap support values (1,000 replicates) are indicated at the nodes. The bar scale indicates genetic distance by using the substitution model of Tamura-Nei [17]

[16]. Tamura-Nei distance was applied for the calculation of genetic distance [17]. Bootstrap re-sampling ($n = 1000$) was performed to evaluate the robustness of the phylogenetic tree [18]. To construct the phylogenetic tree, the nucleotide sequences of the CDS regions in the *ELOVL* family were used (*ELOVL1–7*: human: NM_022821, NM_017770, NM_152310, NM_022726, NM_021814, NM_024090 and NM_001104558, cattle: NM_001034703, NM_001083517, XM_589432, NM_001099050, NM_001046597, NM_00 1102155 and NM_001078042 and mouse: NM_019422, NM_019423, NM_007703, NM_148941, NM_134255, NM_130450 and NM_029001).

Genotyping

Genotyping was carried out by the PCR–RFLP (restriction fragment length polymorphism) method. Two SNPs in the promoter region of *ELOVL5* (g.-204C>G and g.-110T>C) were chosen for genotyping. The PCR was performed as the following: 35 cycles at 94 °C for 30 s, 64 °C for 30 s, 72 °C for 1 min. TaKaRa Ex Taq® Hot Start Version was applied as polymerase. The primer sequences to amplify the region including g.-204C>G were 5′-taaaattgtccagagac acgtgatc-3′ and 5′-gggtatctacaatcagaacg-3′. A mismatch nucleotide (underline) was adopted to introduce a *Taq* I recognition site for the fragment, since no suitable restriction enzyme was detected in the site. The primer sequences for g.-110T>C were 5′-tccagagacacgtgaacca-gagg-3′ and 5′-ccagccaaagttttccggagcc-3′. This product was digested with *Sau96* I. The restriction enzymes were commercially obtained from New England Biolabs (Ipswich, MA).

Statistical analysis

All traits were analyzed by generalized least square method implemented in JMP7 (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) was performed to detect significant factors within a model, which included genotypes of the SNPs and other appropriate factors as fixed effects, i.e. sex, sire and month of slaughter for JB1, and sex and year of slaughter for JB2. While genotypes of g.-110T>C were considered in three models, g.-204C>G was included only for HOL because of biased genotypic frequencies in JB1 and JB2. In addition, all models included age at slaughter as linear and quadratic regressions. Differences between least squares means for genotypes within a gene were examined by Tukey–Kramer's honestly significant difference (HSD) test.

Results

Phyloanalysis among the ELOVL genes

A phylogenic tree was constructed using the nucleotide sequences of the coding regions in the *ELOVL* genes of human, cattle and mouse (Fig. 1). The average values of nucleotide sequence identity between cattle and human and between cattle and mouse were 86.7 and 88.1 %, respectively. Among these species, orthologous genes were clustered into same groups. The *ELOVL* genes were divided into three groups: *ELOVL2*, *5, 4*, *ELOVL1, 7* and *ELOVL3, 6*. Bootstrap support values at the nodes of orthologous genes were 100, suggesting that the *ELOVL* gene family in mammals was functionally differentiated as shown in Fig. 1.

Genotyping

Sequence comparison among four Japanese Black and four Holstein cattle revealed five SNPs in the coding regions of the *ELOVL* genes (*ELOVL1* c.804C>T, *ELOVL2* c.447T>C, *ELOVL3* c.96G>A, *ELOVL5* c.105T>C and *ELOVL5* c.339A>G). All of them were synonymous mutations, suggesting that these SNPs might not influence molecular functions which are supposed to be attributed to conformation of the proteins. Therefore, they were predicted not to affect the economic traits [19]. Subsequently, we investigated DNA polymorphisms in the promoter regions of the

Table 1 Significant test between g.-*110T>C* and the economic traits using ANOVA

Traits	*ELOVL5* g.-110T>C		
	JB1	JB2	HOL
Carcass traits			
Carcass weight	ns	ns	*
Rib-eye area	ns	ns	ns
Rib thickness	ns	ns	ns
Subcutaneous fat thickness	*	ns	*
Yield estimate	**	ns	**
BMS	ns	ns	ns
Fatty acid composition			
C14:0	ns	ns	ns
C14:1	ns	***	ns
C16:0	ns	ns	ns
C16:1	ns	ns	ns
C18:0	ns	ns	ns
C18:1	*	ns	ns
C18:2	ns	ns	ns
MUFA	*	ns	ns
SFA	*	ns	ns
Elongation Index	ns	ns	ns

The perinephric fat tissues of 556 Japanese Black cattle in Miyazaki Prefecture (JB1), the intramuscular fats of the *musculus longissimus thoracis* of 225 Japanese Black cattle from diverse areas in Japan (JB2) and the intramuscular fats of the *diaphragm* of 201 Holstein steers in Tottori Prefecture (HOL) were analyzed

ns Non-significant, *BMS* beef marbling score, *MUFA* monounsaturated fatty acid, *SFA* saturated fatty acid

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

ELOVL5 and the *ELOVL6*. Six polymorphisms were identified in the *ELOVL5* (g.-513C>-, g.-307G>A, g.-306T>G, g.-268G>A g.-204C>G and g.-110T>C), while polymorphisms were not found in the *ELOVL6*.

The proximal promoter sequence located within −200/+80 bp is required for transcriptional activation of mouse *Elovl5* [14]. This region was homologous to −254/−53 bp in cattle, including two of five SNPs (g.-204C>G and g.-110T>C) identified in this study (Fig. 2). Sequence comparison revealed this region was well conserved between cattle and mouse with synteny of 82.8 %, which suggested this region might be involved in the expression of cattle *ELOVL5*. In addition, these SNPs were detected in target sequence of transcription factors. The g.-204C>G was in putative sterol regulatory element binding protein-1 (SREBP-1) binding site, while the g.-110T>C putative aryl hydrocarbon receptor (AhR) binding site. For these reasons, these two SNPs were selected for the association study with carcass traits.

The minor allele of the g.-110T>C was the C allele with frequencies of 0.29 (JB1), 0.43 (JB2) and 0.50 (HOL). Whereas, the frequencies of minor allele G in g.-204C>G were 0.01 in JB1, 0.01 in JB2 and 0.08 in HOL. This SNP was excluded from the association analysis because the limited number of the animals with G allele might prevent successful statistical analysis.

Effect of the polymorphisms in the ELOVL genes on the economic traits

The effect of the g.-110T>C on the carcass traits including fatty acid composition were analyzed by ANOVA (Table 1). The g.-110T>C had significant effects on subcutaneous fat thickness ($P < 0.05$), yield estimate ($P < 0.01$), the percentages of C18:1 ($P < 0.05$), MUFA ($P < 0.05$) and SFA ($P < 0.05$) in JB1, C14:1 ($P < 0.001$) in JB2 and carcass weight ($P < 0.05$), subcutaneous fat thickness ($P < 0.05$) and yield estimate ($P < 0.01$) in HOL.

Honestly significant difference test was conducted to investigate the detailed effects of the g.-110T>C (Table 2). In JB1, subcutaneous fat thickness of animals with T allele (T/T type: 2.39 cm and C/T type: 2.35 cm) was thinner than that of the animals with C/C allele (2.68 cm), leading to lower yield estimate of C/C homozygote. Animals with T/T allele in HOL also had thinner subcutaneous fat (2.01 cm) than those with C/T allele (2.30 cm). Same

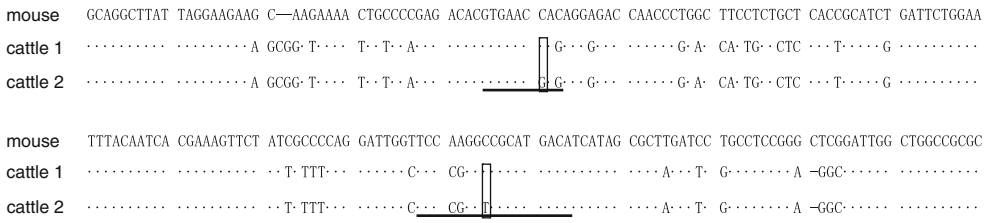

Fig. 2 Two SNPs identified in the promoter region of the cattle *ELOVL5* gene. The promoter region in the cattle *ELOVL5* gene was compared to that in the homologous gene of mouse [14]. The region was well conserved between the two species. Two SNPs were identified in this region (g.-204C>G and g.-110T>C). The g.-204C>G lay in putative sterol regulatory element binding protein-1 (SREBP-1) binding site, while the g.-110T>C putative aryl hydrocarbon receptor (AhR) binding site. The identified SNPs are indicated as the boxed nucleotides and putative transcription factor binding sites as underlined sequences. Dots indicate the nucleotides identical between cattle and mouse, and hyphens in/del polymorphisms

tendency was observed in JB2, though this group did not show significance. Moreover, animals with T/T type in JB2 showed higher percentage of C14:1 than the others, and C/C type in HOL indicated greater carcass weight than the others.

Discussion

Orthologous genes among three species were clustered into same groups and the phylogenic relationship of the ELOVL family was identical with their substrate preferences (Fig. 1). The substrates of ELOVL2, 4 and 5 were polyunsaturated fatty acids, while those of the others were SFA and MUFA [5, 8]. This suggested that an ancestral enzyme of the ELOVL family preferred SFA and MUFA, and ELOVL2, 4 and 5 gained new characteristic during the evolution.

Polymorphism search in CDS regions revealed that nonsynonymous mutations were not identified in the *ELOVL* genes. In addition, the CDS regions in the *ELOVL* genes were well conserved among human, cattle and mouse. Aberrant activities of the ELOVL family have been observed in various human diseases such as macular degeneration, neurological disorder, cancer and diabetes [8, 20–22]. These data suggested the importance of the ELOVL family in fatty acid elongation and functional alteration of the ELOVL family may be too influential for living organisms to keep sound homeostasis of fatty acids.

The region around two SNPs of the promoter region in the *ELOVL5* was well conserved between cattle and mouse (Fig. 2). Qin et al. [14] reported that this region was essential for transcriptional activation of mouse *Elovl5*. They also identified the sterol regulatory element 1 (SRE-1) in this region. The SRE-1 is the target sequence of sterol regulatory element binding protein-1 (SREBP-1) [23], suggesting the expression of the *ELOVL5* gene was regulated by the SREBP-1. Hoashi et al. [24] elucidated that an in/del polymorphism in intron 5 of the *SREBP-1* gene had a significant effect on fatty acid composition in beef cattle. The expression of the *SREBP-1* and its related genes including the *ELOVL5* might be an important factor to influence fatty acid composition. The effect of the g.-110T>C on the carcass traits might result from the alteration of the *ELOVL5* expression.

Our result revealed that the g.-110T>C had a significant effect on subcutaneous fat thickness (Tables 1, 2). Animals with T allele tended to have thinner subcutaneous fat and, therefore, exhibit better yield estimate. Subcutaneous fat is mainly composed of triacylglycerol [25]. Moon et al. [6] reported that deletion of the *ELOVL5* led to accumulation of triacylglycerol through activation of SREBP-1 in mice. The C allele of the g.-110T>C might suppress the

Table 2 Effect of the genotypes for the *ELOVL5*g.-110T>C on the economic traits in the three cattle groups

Traits	JB1			JB2			HOL		
	T/T ($n = 277$)	C/T ($n = 232$)	C/C ($n = 47$)	T/T ($n = 91$)	C/T ($n = 84$)	C/C ($n = 50$)	T/T ($n = 51$)	C/T ($n = 100$)	C/C ($n = 50$)
Carcass traits									
CW (kg)	427.49 ± 4.15	427.29 ± 4.38	426.49 ± 7.37	421.66 ± 5.41	433.06 ± 5.55	437.89 ± 6.59	453.23 ± 6.00^{b}	455.13 ± 4.34^{b}	470.85 ± 5.00^{a}
SFT (cm)	2.39 ± 0.06^{b}	2.35 ± 0.07^{b}	2.68 ± 0.11^{a}	2.92 ± 0.09	3.00 ± 0.09	3.10 ± 0.11	2.01 ± 0.10^{b}	2.30 ± 0.07^{a}	2.26 ± 0.08^{ab}
YE (%)	74.15 ± 0.12^{ab}	74.40 ± 0.12^{a}	73.81 ± 0.21^{b}	73.87 ± 0.16	73.89 ± 0.16	73.37 ± 0.19	69.75 ± 0.17^{a}	69.29 ± 0.12^{b}	69.15 ± 0.14^{b}
Fatty acid composition (%)									
C14:1	0.91 ± 0.03	0.90 ± 0.03	0.91 ± 0.05	0.96 ± 0.03^{a}	0.80 ± 0.03^{b}	0.84 ± 0.04^{b}	0.62 ± 0.03	0.59 ± 0.02	0.60 ± 0.03

The perinephric fat tissues of 556 Japanese Black cattle in Miyazaki Prefecture (JB1), the intramuscular fats of the *musculus longissimus thoracis* of 225 Japanese Black cattle from diverse areas in Japan (JB2) and the intramuscular fats of the *diaphragm* of 201 Holstein steers in Tottori Prefecture (HOL) were analyzed. Values are least square means with standard error

CW carcass weight, *SFT* subcutaneous fat thickness, *YE* yield estimate

[a, b] Means with different superscripts within the same trait and group differ significantly at $p < 0.05$ (Tukey's HSD analysis)

expression of the *ELOVL5* to produce triacylglycerol in subcutaneous fat, leading to thicker subcutaneous fat (Table 2). The g.-110T>C had also significant effects on the percentage of C14:1 in JB2 and on carcass weight in HOL (Table 2). However, these effects were not common among the groups and stable tendencies were not observed. The difference of genetic background among the groups might explain these mismatched results. Breed difference is an important factor to influence fatty acids in beef when fed under same conditions [26, 27].

The g.-110T>C might influence aryl hydrocarbon receptor (AhR) binding. The T allele of the g.-110T>C produced putative AhR binding site, while the other did not. The AhR is a ligand-activated transcription factor, which represses several enzymes in fatty acid synthesis pathway including FASN and serum triglyceride levels in mice [28]. Thicker subcutaneous fat of animals with C/C allele might result from the absence of AhR binding site. The effect of the g.-110T>C on the *ELOVL5* expression needs to be elucidated in the future.

The g.-204C>G was located within the SRE-1 sequence (Fig. 2), suggesting this SNP altered the *ELOVL5* expression. Although this SNP did not significantly affect any carcass traits analyzed in this study due to the limited number of the animals with G allele, more detailed study about this SNP will be needed in the future. Elucidating the effect of this SNP on the expression of the *ELOVL5* gene will provide new insights for the association between economic traits in beef with genes which expressions are governed by the SREBP-1.

In this study, we performed DNA polymorphism search in the CDS and promoter regions of the *ELOVL* genes. As a result, five synonymous mutations were detected in the CDS regions of the *ELOVL* genes and six polymorphisms in the promoter region of the *ELOVL5*. The SNP in the promoter region of the *ELOVL5* (g.-110T>C) was expected to alter the expression of this gene and effective on the plural economic traits including subcutaneous fat thickness. The g.-110T>C will be a useful genetic marker for the breeding of beef cattle.

Acknowledgments This study was supported in part by a Research Grant from Livestock Improvement Promotion Project in Ministry of Agriculture, Forestry and Fisheries.

References

1. Ibeagha-Awemu EM, Kgwatalala P, Zhao X (2008) A critical analysis of production-associated DNA polymorphisms in the genes of cattle, goat, sheep, and pig. Mamm Genome 19:591–617. doi:10.1007/s00335-008-9141-x
2. Taniguchi M, Utsugi T, Oyama K, Mannen H, Kobayashi M, Tanabe Y, Ogino A, Tsuji S (2004) Genotype of *stearoyl-CoA desaturase* is associated with fatty acid composition in Japanese Black cattle. Mamm Genome 15:142–148. doi:10.1007/s00335-003-2286-8
3. Michal JJ, Zhang ZW, Gaskins CT, Jiang Z (2006) The bovine *fatty acid binding protein 4* gene is significantly associated with marbling and subcutaneous fat depth in Wagyu × Limousin F_2 crosses. Anim Genet 37:400–402. doi:10.1111/j.1365-2052.2006.01464.x
4. Bonet ML, Ribot J, Palou A (2012) Lipid metabolism in mammalian tissues and its control by retinoic acid. Biochim Biophys Acta 1821:177–189. doi:10.1016/j.bbalip.2011.06.001
5. Jakobsson A, Westerberg R, Jacobsson A (2006) Fatty acid elongases in mammals: their regulation and roles in metabolism. Prog Lipid Res 45:237–249. doi:10.1016/j.plipres.2006.01.004
6. Moon YA, Hammer RE, Horton JD (2009) Deletion of *ELOVL5* leads to fatty liver through activation of SREBP-1c in mice. J Lipid Res 50:412–423. doi:10.1194/jlr.M800383-JLR200
7. Matsuzaka T, Shimano H, Yahagi N, Yoshikawa T, Amemiya-Kudo M, Hasty AH, Okazaki H, Tamura Y, Iizuka Y, Ohashi K, Osuga J, Takahashi A, Yato S, Sone H, Ishibashi S, Yamada N (2002) Cloning and characterization of a mammalian *fatty acyl-CoA elongase* as a lipogenic enzyme regulated by SREBPs. J Lipid Res 43:911–920
8. Tamura K, Makino A, Hullin-Matsuda F, Kobayashi T, Furihata M, Chung S, Ashida S, Miki T, Fujioka T, Shuin T, Nakamura Y, Nakagawa H (2009) Novel lipogenic enzyme ELOVL7 is involved in prostate cancer growth through saturated long-chain fatty acid metabolism. Cancer Res 69:8133–8140. doi:10.1158/0008-5472.CAN-09-0775
9. Green CD, Ozguden-Akkoc CG, Wang Y, Jump DB, Olson LK (2010) Role of fatty acid elongases in determination of *de novo* synthesized monounsaturated fatty acid species. J Lipid Res 51:1871–1877. doi:10.1194/jlr.M004747
10. Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:487–509
11. Morris CA, Cullen NG, Glass BC, Hyndman DL, Manley TR, Hickey SM, McEwan JC, Pitchford WS, Bottema CD, Lee MA (2007) *Fatty acid synthase* effects on bovine adipose fat and milk fat. Mamm Genome 18:64–74. doi:10.1007/s00335-006-0102-y
12. O'Keefe PW, Wellington GH, Mattick LR, Stouffer JR (1968) Composition of bovine muscle lipids at various carcass locations. J Food Sci 33:188–192
13. Kumadaki S, Matsuzaka T, Kato T, Yahagi N, Yamamoto T, Okada S, Kobayashi K, Takahashi A, Yatoh S, Suzuki H, Yamada N, Shimano H (2008) Mouse *Elovl-6* promoter is an SREBP target. Biochem Biophys Res Commun 368:261–266. doi:10.1016/j.bbrc.2008.01.075
14. Qin Y, Dalen KT, Gustafsson JA, Nebb HI (2009) Regulation of hepatic fatty acid elongase 5 by LXRα–SREBP-1c. Biochim Biophys Acta 1791:140–147. doi:10.1016/j.bbalip.2008.12.003
15. Heinemeyer T, Wingender E, Reuter I, Hermjakob H, Kel AE, Kel OV, Ignatieva EV, Ananko EA, Podkolodnaya OA, Kolpakov FA, Podkolodny NL, Kolchanov NA (1998) Databases on transcriptional regulation: TRANSFAC, TRRD and COMPEL. Nucleic Acids Res 26:362–367. doi:10.1093/nar/26.1.362
16. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599. doi:10.1093/molbev/msm092
17. Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526
18. Efron B (1982) The jackknife, the bootstrap, and other resampling plans. CBMS-NSF regional conference series in applied mathematics No.38. Cambridge University Press, Cambridge

19. Chang X, Qin Y, Xu C, Li G, Zhao X, Chen ZJ (2012) Mutations in *WNT4* are not responsible for Müllerian duct abnormalities in Chinese women. Reprod Biomed Online 24:630–633. doi:10.1016/j.rbmo.2012.03.008
20. Zhang K, Kniazeva M, Han M, Li W, Yu Z, Yang Z, Li Y, Metzker ML, Allikmets R, Zack DJ, Kakuk LE, Lagali PS, Wong PW, MacDonald IM, Sieving PA, Figueroa DJ, Austin CP, Gould RJ, Ayyagari R, Petrukhin K (2001) A 5-bp deletion in *ELOVL4* is associated with two related forms of autosomal dominant macular dystrophy. Nat Genet 27:89–93. doi:10.1038/83817
21. Matsuzaka T, Shimano H, Yahagi N, Kato T, Atsumi A, Yamamoto T, Inoue N, Ishikawa M, Okada S, Ishigaki N, Iwasaki H, Iwasaki Y, Karasawa T, Kumadaki S, Matsui T, Sekiya M, Ohashi K, Hasty AH, Nakagawa Y, Takahashi A, Suzuki H, Yatoh S, Sone H, Toyoshima H, Osuga J, Yamada N (2007) Crucial role of a long-chain fatty acid elongase, Elovl6, in obesity-induced insulin resistance. Nat Med 13:1193–1202. doi:10.1038/nm1662
22. Lee YJ, Wang S, Slone SR, Yacoubian TA, Witt SN (2011) Defects in *very long chain fatty acid synthesis* enhance alpha-synuclein toxicity in a yeast model of Parkinson's disease. PLoS ONE 6:e15946. doi:10.1371/journal.pone.0015946
23. Yokoyama C, Wang X, Briggs MR, Admon A, Wu J, Hua X, Goldstein JL, Brown MS (1993) SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. Cell 75:187–197. doi:10.1016/S0092-8674(05)80095-9
24. Hoashi S, Ashida N, Ohsaki H, Utsugi T, Sasazaki S, Taniguchi M, Oyama K, Mukai F, Mannen H (2007) Genotype of bovine *sterol regulatory element binding protein-1* (*SREBP-1*) is associated with fatty acid composition in Japanese Black cattle. Mamm Genome 18:880–886. doi:10.1007/s00335-007-9072-y
25. Yang A, Larsen TW, Powell VH, Tume RK (1999) A comparison of fat composition of Japanese and long-term grain-fed Australian steers. Meat Sci 51:1–9. doi:10.1016/S0309-1740(98)00065-5
26. Matsuzaki M, Takizawa S, Ogawa M (1997) Plasma insulin, metabolite concentrations, and carcass characteristics of Japanese Black, Japanese Brown, and Holstein steers. J Anim Sci 75:3287–3293
27. Albrecht E, Gotoh T, Ebara F, Xu JX, Viergutz T, Nürnberg G, Maak S, Wegner J (2011) Cellular conditions for intramuscular fat deposition in Japanese Black and Holstein steers. Meat Sci 89:13–20. doi:org/10.1016/j.meatsci.2011.03.012
28. Tanos R, Murray IA, Smith PB, Patterson A, Perdew GH (2012) Role of the ah receptor in homeostatic control of fatty acid synthesis in the liver. Toxicol Sci 129:372–379. doi:10.1093/toxsci/kfs204