https://doi.org/10.17221/105/2016-CJAS

Alternative Polyadenylation and Polymorphisms of 3'Untranslated Regions of Bovine *BBOX1* Gene

*Guo-li Zhou[1], Yang Cao[2], You-zhi Xin[1], Yu-fei Song[1], Hai-guo Jin[2]**

[1]*College of Life Science, Liaocheng University, Liaocheng, P.R. China*

[2]*Branch of Animal Husbandry, Jilin Academy of Agricultural Sciences, Gongzhuling, P.R. China*

**Corresponding author: khk1962@126.com*

ABSTRACT

Zhou G.-L., Cao Y., Xin Y.-Z., Song Y.-F., Jin H.-G. (2018): **Alternative polyadenylation and polymorphisms of 3'untranslated regions of bovine *BBOX1* gene**. Czech J. Anim. Sci., 63, 188–194.

L-Carnitine, a key element in fatty acid metabolism and energy production, is biosynthesized from gamma-butyrobetaine by the catalysis of gamma-butyrobetaine hydroxylase (BBOX1). We cloned three different 3'untranslated regions (3'UTRs) alternative polyadenylation (APA) transcripts of the *BBOX1* gene with different 3'UTR length (GenBank Accession Nos. KX431577, KX431578, KX431579). Two polymorphisms, NM_001101881.2: g.1797_1798insTGC and g.1935T>C, were revealed in 3'UTR of *BBOX1* gene. They created or disrupted a restriction site for endonuclease *Bbv*I and *Hinc*II, respectively. Moreover, the single nucleotide polymorphism (SNP) g.1935T>C can create or disrupt polyadenylation signals PAS3 resulting in the presence of APA3 transcript variant. Marker-trait association analyses showed that the *BBOX1-Bbv*I and *BBOX1-Hinc*II loci were significantly associated with muscle fibre diameter, shear force, net meat weight, and carcass weight ($P < 0.01$). Moreover, we also found a significant association of combined genotypes with cooking loss, muscle fibre diameter, shear force, net meat weight, and carcass weight ($P < 0.01$). The results of this study provide the evidence that polymorphisms in *BBOX1* gene are associated with meat quality and carcass traits in Chinese Red cattle, and may be used as a candidate for marker assisted selection in beef cattle breeding program.

Keywords: candidate gene; transcript variants; meat quality; carcass traits; association analysis

Gamma-butyrobetaine hydroxylase (BBOX1) is an enzyme responsible for the biosynthesis of L-carnitine, a key molecule of fatty acid metabolism. There are four enzymatic reactions for the synthesis of L-carnitine, and the last step is catalyzed by BBOX1. The enzyme activity of BBOX1 in human has been detected only in the liver, kidneys, and brain (Rigault et al. 2006). Increasing evidence indicates that L-carnitine deficiency may be implicated in obesity (Cave et al. 2008). It has been shown that carnitine supplementation can improve glucose tolerance in obese rats. More-over, modulation of carnitine palmitoyltransferase I activity also contributes to the changes in lipid metabolism and food intake (Lopaschuk et al. 2010). Therefore, L-carnitine might play a key role in regulating fatty acid and energy metabolism.

Recent genomic analyses revealed that most eukaryotic genes have multiple polyadenylation sites, resulting in transcript variants with different coding potentials and/or variable 3'UTR. Moreover, the alternative polyadenylation (APA) is an important layer of gene regulation affecting mRNA metabolism (Tian and Manley 2013). The

Supported by the National Natural Science Foundation of China (No. 31571274) and the Natural Science Fund of Shandong province of P.R. China (ZR2015CM025) and Platform of Domestic Animal Germplasm Resources.

https://doi.org/10.17221/105/2016-CJAS

human *BBOX1* cDNA consists of an 1161 bp open reading frame encoding a polypeptide of 387 amino acids. Two 3'UTR with different length were generated by an APA mechanism during the human *BBOX1* mRNA maturation (Rigault et al. 2006). Four alternative polyadenylated *BBOX1* mRNAs were identified in rat liver. Moreover, the level of three *BBOX1* mRNA transcript variants in rat liver was selectively increased by high fat diet and the alternative use of polyadenylation sites contributed to the global increase in BBOX1 enzymatic activity and L-carnitine levels. A selective polyadenylation process was found to nutritionally regulate the maturation of *BBOX1* mRNAs in liver to adjust biosynthesis of L-carnitine to the energy supply (Rigault et al. 2013).

The bovine *BBOX1* gene could be considered as a candidate gene associated with meat quality and carcass traits due to its significance in fatty acid and energy metabolism. However, organization and polymorphisms of the bovine *BBOX1* gene have not been well investigated yet. The purpose of this study was to investigate the alternative polyadenylation and polymorphisms in the 3'UTR of bovine *BBOX1* gene and the relationship between polymorphisms and meat quality and carcass traits of a Chinese native cattle breed.

MATERIAL AND METHODS

Ethics statement. All procedures involving animals were approved by the Animal Care and Use Committee at the Institute of Jilin Academy of Agricultural Sciences where the experiment was conducted, and also approved and authorized by the Chinese Ministry of Agriculture.

Animals, genomic DNA and total RNA isolation. A total of 307 Chinese Red cattle were randomly selected between 2010 and 2013 from Branch of Animal Husbandry, Jilin Academy of Agricultural Sciences, China for use in the association analysis. They were the progeny of five sires and unrelated dams (n = 58, 68, 53, 66, and 62 progenies per sire, respectively). Age of animals at slaughter ranged from 24 to 26 months. All carcass and meat quality traits were measured according to the criterion GB/T 17238-1998 of the Cutting Standard of Fresh and Chilled Beef in China (China Standard Publishing House), and also according to reference methods described by Prieto et al. (2008) and Allais et al. (2010, 2011). Carcass measurements including net meat weight, carcass weight, and rib eye area were obtained at postmortem. The *longissimus lumborum* samples were obtained between the 11th and 13th ribs collected at postmortem, placed in a labelled vacuum-packed bag in a cooler, and transported to the laboratory for collection of meat quality phenotypes data.

Genomic DNA was extracted from whole blood by phenol-chloroform method, and then dissolved in Tris-EDTA (TE) buffer and kept at –20°C and/or at 4°C. Total RNA was isolated from liver, kidney, heart, rumen and *longissimus lumborum* tissues using the RNeasy kit (Qiagen, Germany). RNA integrity was monitored by denaturing 1% agarose gel electrophoresis. Concentrations and purities of RNA were measured by spectrophotometry (Amersham Pharmacia Biotech, UK).

Identification of alternative polyadenylation by 3'RACE. The 3'RACE (rapid amplification of cDNA ends) experiment was performed using the First-Choice® RLM-RACE Kit (Thermo Fisher Scientific, USA) according to the manufacturer's recommendations on total RNA extracted. Primers for 3'RACE were designed based on the sequence of bovine *BBOX1* gene (GenBank Accession No. NM_001101881.2). The outer bovine *BBOX1* gene specific forward primer GSP (5'-GGAACTGAGATAACCCGCCA-3') was used in combination with the adaptor specific outer primer for the first polymerase chain reaction (PCR) amplification. The bovine *BBOX1* gene specific inner primer NGSP (5'-GGCTTATGCTGACTGGGATGTG-3') was used in combination with the adaptor specific inner primer for the second PCR. The nested PCR products were analysed on 2% agarose gel and sequenced to identify the 3' end of the targeted mRNA.

Polymorphisms identification and genotyping. For polymorphisms identification and genotyping of *BBOX1* 3'UTR, primers were designed based on the sequence of bovine *BBOX1* gene (GenBank Accession No. NM_001101881.2) and its 3' flanking region (Table 1). Chinese Red steers (n = 30) were randomly chosen for screening DNA polymorphisms using polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) method. Briefly, PCR were carried out in 25 µl volume containing 0.2 µmol/l each primer, 1× PCR buffer, 0.2 mmol/l each dNTP, 50 ng genomic DNA, 1.0 Unit *Taq* DNA polymerase, and the rest was ddH_2O. The Mg^{2+}concentration was optimized

https://doi.org/10.17221/105/2016-CJAS

for each primer set (Table 1). The PCR conditions were at 94°C for 5 min, followed by 32 cycles of 94°C for 30 s, annealing at an optimal annealing temperature (Table 1) for each primer for 30 s and at 72°C for 40 s, with a final extension at 72°C for 10 min.

The SSCP analysis was used to identify the polymorphisms. Briefly, PCR products were half diluted in denaturing loading dye (95% formamide, 0.025% bromophenol blue, and 0.025% xylene cyanol), denatured at 95°C for 10 min, and then placed on ice for 10 min. The samples were then loaded on 10% nondenaturing polyacrylamide gel with 10% formamide, and run in 1×Tris-borate-EDTA (TBE) buffer at 180 V for 14–16 h at a constant temperature of 4°C. The gel was stained with 0.1% silver nitrate. The PCR products of different electrophoresis patterns were purified with Axygen kits (MBI Fermentas, Lithuania) and were sent to Sangon Biotech (Shanghai) Co., Ltd. for sequencing in both directions in an ABI PRIZM 377 DNA sequencer (Perkin-Elmer, USA). The sequences were analyzed with DNAstar software (Version 5.01).

All polymorphisms identified by PCR-SSCP and sequencing were confirmed by the PCR-restriction fragment length polymorphism (RFLP) method using relevant restriction enzymes from New England Biolabs (NEB, China) (Table 1). They also were genotyped by the PCR-RFLP method in a total of 307 Chinese Red steers. The reaction mixture comprised 5 µl PCR product, 2 µl 10× buffer, 0.5 µl (5 U) restriction enzymes, and 12.5 µl ddH_2O. The digestion products were subjected to horizontal electrophoresis (90 V, 30 min) on a 3.0% (w/v) agarose in 1× Tris-acetate-EDTA (TAE) buffer and were stained with ethidium bromide.

Statistical analyses. Genotype frequencies of *BBOX1-Bbv*I and *BBOX1-Hin*cII loci were calculated directly from the genotypes of the 307 cattle, respectively. The linkage disequilibrium (LD) structure as measured by D' and r^2 was computed with the HAPLOVIEW software (Version. 3.32) (Barrett et al. 2005). Haplotype frequencies were analyzed by PHASE (Version. 2.1.1) (Stephens et al. 2001). The associations between genotypes, combined genotypes, and carcass and meat quality traits were evaluated using the Least Squares method (GLM procedure of the SAS software – Statistical Analysis System, Version 8.01, 1999). Duncan's multiple range tests from PROC GLM were used to separate means. $P < 0.01$ was considered significant. The statistical model used was as follows:

$$Y_{ijkl} = \mu + G_i + S_j + YS_k + bD_l + e_{ijkl}$$

where:

Y_{ijkl} = observation of the meat quality traits
μ = overall mean for each trait
G_i = fixed effect of the i^{th} genotype or the i^{th} combined genotypes
S_j = effect of the j^{th} sire
YS_k = effect of the k^{th} seasons of slaughter
b = regression coefficient for slaughter age in days
D_l = slaughter age in days
e_{ijkl} = random residual error

Least Squares Means and standard errors for carcass and meat quality traits were estimated for

Table 1. Polymerase chain reaction (PCR) primers and amplification conditions for identification of polymorphisms in *BBOX1* gene

Primer name	Primer sequences	Size (bp)	T_a (°C)	Mg^{2+} (mmol/l)	Primer purpose
UTR1	5'-ATGCTGACTGGGATGTGG-3' 5'-GGGCAAAAGAGAGTTCAGGAT-3'	623	60	2.0	PCR-SSCP and PCR-RFLP (*Bbv*I)
UTR2	5'-GAAATGAATCCGCCACAGGTAT-3' 5'-TTGGTGAGGGCTGGAAAT-3'	365	58	1.5	PCR-SSCP
ACRS[1]	5'-GCTTCTTTTGAATAAAGCTTTGTGTCA-3' 5'-TTGGTGAGGGCTGGAAAT-3'	243	57	2.0	PCR-RFLP (*Hin*cII)

T_a = annealing temperature, PCR-SSCP = polymerase chain reaction-single strand conformation polymorphism, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, UTR = untranslated region, ACRS = amplification-created restriction sites

[1]forward mismatched primer designed with web-based program dCAPS Finder 2.0 (http://helix.wustl.edu/dcaps/dcaps.html); mismatched base was underlined; single nucleotide polymorphism g.1935T>C identified was confirmed by digestion of corresponding PCR products with restriction endonuclease *Hin*cII

https://doi.org/10.17221/105/2016-CJAS

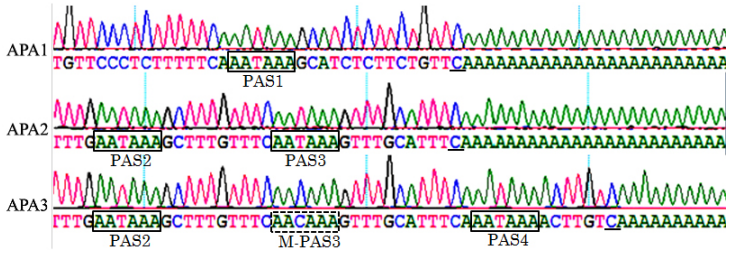

Figure 1. The part 3' sequences and peak map of three different 3' untranslated regions (3'UTRs) alternative polyadenylation (APA) transcript variants of *BBOX1* gene. Three different 3'UTRs APA transcript variants are named as APA1, APA2, and APA3. The predicted canonical polyadenylation signals "AATAAA" in solid line rectangular areas are numbered from PAS1 to PAS4. The sequence "AACAAA" in a broken line rectangular area indicates PAS3 mutated occurs only in APA3. The different polyadenylation sites identified by our 3'RACE experiments are underlined

genotypes of *BBOX1-Bbv*I, *BBOX1-Hin*cII loci, and the combined genotypes.

RESULTS

The 3'UTR of BBOX1 involves alternative polyadenylation. In order to detect as many APA transcript variants of *BBOX1* as possible, 3'RACE was performed on RNA isolated from bovine liver, kidneys, heart, rumen, and *longissimus lumborum* tissues. But three different 3'UTRs APA transcript variants of *BBOX1* (APA1, APA2, and APA3) were only detected in bovine liver and kidneys tissue. The length of 3'UTRs APA1 transcript variant by proximal polyadenylation signals PAS1 choice is 229 bp, while the lengths of the other two 3'UTRs APA2 and APA3 transcript variants with longer 3'UTRs by distal polyadenylation signals PAS3 and PAS4 choice are 664 bp and 678 bp, respectively. When this PAS3 "AATAAA" was mutated into "AACAAA" (NM_001101881.2:g.1935T>C), a shift of APA site was observed resulting in the presence of APA3 transcript variant by usage of PAS4 (Figure 1). The sequences of the 3'UTR identified by 3'RACE have been submitted to GenBank and their Accession Nos. are KX431577, KX431578, and KX431579, respectively.

Polymorphisms of the 3'UTR in BBOX1 gene. Two polymorphisms, NM_001101881.2: g.1797_1798insTGC and g.1935T>C, were revealed by PCR-SSCP and sequencing in the 3'UTR of *BBOX1* gene, and they create or disrupt restriction site for endonuclease *Bbv*I and *Hin*cII, respectively. The *BBOX1-Bbv*I and *BBOX1-Hin*cII loci were genotyped by the PCR-RFLP method in the studied population. The following DNA restriction fragments were obtained for the *BBOX1-Bbv*I poly-

Table 2. Least Squares Means and standard errors for meat quality and carcass traits in Chinese Red cattle according to *BBOX1-Bbv*I and *BBOX1-Hin*cII genotypes

Traits	*BBOX1-Bbv*I genotypes			*BBOX1-Hin*cII genotypes		
	NN (*n* = 194)	*NI* (*n* = 82)	*II* (*n* = 31)	*TT* (*n* = 166)	*TC* (*n* = 108)	*CC* (*n* = 33)
Cooking loss (%)	40.13 ± 10.20	39.54 ± 10.31	39.15 ± 10.50	39.75 ± 10.22	39.94 ± 10.27	40.29 ± 10.49
Muscle fibre diameter (μm)	33.13 ± 10.24^{b}	33.80 ± 10.37^{b}	37.18 ± 10.60^{a}	33.35 ± 10.26^{b}	33.29 ± 10.32^{b}	36.99 ± 10.59^{a}
Shear force (N/cm^2)	36.25 ± 10.38^{a}	33.78 ± 10.58^{b}	37.66 ± 10.94^{a}	36.56 ± 10.40^{a}	33.91 ± 10.50^{b}	37.57 ± 10.91^{a}
Drip loss (%)	2.40 ± 10.06	2.60 ± 10.10	2.27 ± 10.16	2.42 ± 10.07	2.56 ± 10.09	2.14 ± 10.15
pH_{24h}	5.57 ± 10.009	5.57 ± 10.014	5.55 ± 10.023	5.57 ± 10.010	5.58 ± 10.012	5.58 ± 10.022
Net meat weight (kg)	269.4 ± 11.85^{ab}	261.0 ± 12.85^{b}	278.0 ± 14.62^{a}	267.3 ± 12.01^{ab}	265.4 ± 12.50^{b}	280.2 ± 14.54^{a}
Carcass weight (kg)	318.0 ± 11.90^{a}	307.7 ± 12.94^{b}	325.3 ± 14.75^{a}	315.8 ± 12.08^{ab}	312.6 ± 12.57^{b}	328.6 ± 14.70^{a}
Rib eye area (cm^2)	90.6 ± 10.94	89.0 ± 11.45	87.2 ± 12.34	90.7 ± 11.01	89.1 ± 11.26	88.0 ± 12.29

pH_{24h} = pH measured 24 h post-mortem

$^{a, b}$values with different superscripts in the same row significantly differ ($P < 0.01$)

https://doi.org/10.17221/105/2016-CJAS

morphism: 623 bp for the *NN* genotype; 623, 570, and 56 bp for the *NI* genotype, and 570 and 56 bp for the *II* genotype. The *BBOX1-Bbv*I *NN* genotype had the highest frequency in the herds studied (0.633), followed by the *NI* genotype (0.266). The least frequent genotype was *II* (0.101). The DNA restriction fragments obtained for the *BBOX1-Hin*cII polymorphism: 243 bp for the *TT* genotype; 243, 217, and 26 bp for the *TC* genotype; and 217 and 26 bp for the *CC* genotype. The *BBOX1-Hin*cII *TT* genotype had the highest frequency in the herds studied (0.541), followed by the *TC* genotype (0.353). The least frequent genotype was *CC* (0.106).

To reveal the linkage relationships between *BBOX1-Bbv*I and *BBOX1-Hin*cII loci, linkage disequilibrium between these two loci was estimated. The *BBOX1-Bbv*I and *BBOX1-Hin*cII were weakly linked (r^2 = 0.028, D' = 0.191) indicating that these SNPs were having little linkage disequilibrium. The haplotype analysis showed that four different haplotypes (H1 (*NT*), H2 (*NC*), H3 (*IT*), and H4 (*IC*)) were identified between *BBOX1-Bbv*I and *BBOX1-Hin*cII loci. Frequencies of the four haplotypes were 0.581, 0.184, 0.136, and 0.098, respectively.

Association analysis. The loci of *BBOX1-Bbv*I and *BBOX1-Hin*cII were genotyped by PCR-RFLP in 307 Chinese Red cattle, in order to investigate the association of *BBOX1* gene polymorphisms with meat quality and carcass traits. The results of the association analyses between single markers and meat quality and carcass traits are shown in Table 2. Marker-trait association analyses showed that the *BBOX1-Bbv*I and *BBOX1-Hin*cII loci were significantly associated with muscle fibre diameter, shear force, net meat weight, and carcass weight ($P < 0.01$). In addition, the combined genotypes association results for *BBOX1-Bbv*I and *BBOX1-Hin*cII loci are shown in Table 3. The results of association analyses indicated that the combined genotypes were significantly associated with cooking loss, muscle fibre diameter, shear force, net meat weight, and carcass weight in the Chinese Red cattle population ($P < 0.01$).

Table 3. Associations between combined genotypes of *BBOX1-Bbv*I and *BBOX1-Hin*cII and meat quality and carcass traits in Chinese red cattle

Combined genotypes	Number	Cooking loss (%)	Muscle fibre diameter (μm)	Shear force (N/cm^2)	Drip loss (%)	pH_{24h}	Net meat weight (kg)	Carcass weight (kg)	Rib eye area (cm^2)
NN-TT	116	39.82 ± 10.26^{ab}	33.03 ± 10.30^{bc}	37.12 ± 10.47^{ab}	2.37 ± 10.82	5.57 ± 10.01	269.0 ± 12.39^{ab}	317.9 ± 12.45^{ab}	90.7 ± 11.22
NN-TC	64	40.38 ± 10.35^{ab}	32.64 ± 10.40^{c}	34.52 ± 10.63^{ab}	2.49 ± 10.11	5.59 ± 10.02	268.3 ± 13.21^{ab}	316.1 ± 13.30^{ab}	90.5 ± 11.65
NI-TT	37	39.55 ± 10.46^{ab}	33.33 ± 10.52^{bc}	34.13 ± 10.83^{ab}	2.63 ± 10.15	5.59 ± 10.02	259.9 ± 14.21^{b}	306.8 ± 14.33^{b}	91.6 ± 12.16
NI-TC	35	39.63 ± 10.47^{ab}	33.50 ± 10.53^{bc}	32.34 ± 10.85^{b}	2.69 ± 10.15	5.57 ± 10.02	257.8 ± 14.30^{b}	304.2 ± 14.42^{b}	87.5 ± 12.20
NN-CC	15	41.48 ± 10.72^{a}	35.97 ± 10.83^{ab}	36.81 ± 11.31^{ab}	2.22 ± 10.23	5.52 ± 10.03	276.5 ± 16.67^{ab}	326.2 ± 16.85^{ab}	91.0 ± 13.41
NI-CC	9	39.14 ± 10.93^{ab}	36.96 ± 11.07^{a}	38.01 ± 11.70^{a}	2.12 ± 10.30	5.46 ± 10.04	278.1 ± 18.60^{ab}	325.5 ± 18.84^{ab}	84.6 ± 14.41
II-TT	13	39.67 ± 10.76^{ab}	36.21 ± 10.87^{ab}	38.46 ± 11.39^{a}	2.29 ± 10.24	5.52 ± 10.04	273.2 ± 17.02^{ab}	321.6 ± 17.22^{ab}	88.5 ± 13.60
II-TC	9	38.07 ± 10.93^{b}	37.11 ± 11.07^{a}	35.73 ± 11.70^{ab}	2.50 ± 10.30	5.50 ± 10.04	274.6 ± 18.60^{ab}	320.6 ± 18.84^{ab}	86.0 ± 14.40
II-CC	9	39.44 ± 10.93^{ab}	38.71 ± 11.07^{a}	38.38 ± 11.70^{a}	2.01 ± 10.30	5.46 ± 10.04	288.4 ± 18.60^{a}	335.6 ± 18.84^{a}	86.3 ± 14.40

pH_{24h} = pH measured 24 h post-mortem

$^{a-c}$values with different superscripts in the same column significantly differ ($P < 0.01$)

DISCUSSION

Recent genomic studies have indicated that APA exists in 70–79% of mammalian genes. Moreover, a great majority of yeast and plant genes have also

https://doi.org/10.17221/105/2016-CJAS

been reported to show APA (Tian and Manley 2013). In the present study, three different 3'UTRs APA transcript variants were detected in bovine *BBOX1* gene. These results indicate that the size differences of the three 3'UTRs of *BBOX1* mRNA are due to heterogeneity at the 3'UTR that originates from the use of alternative polyadenylation signals. Moreover, from our results of 3'RACE and sequencing, we speculate that the polyadenylation signals PAS2 might not be functional, and further studies are needed to investigate the potential role of PAS2 in the regulation of mRNA processing. In human, two cleavage sites were identified to generate a short and a long 3'UTR in the three investigated organs (Rigault et al. 2006). In addition, four different 3'UTRs were identified on rat liver mRNAs (Rigault et al. 2013).

The present study is the first report on polymorphisms of the bovine *BBOX1* gene. Two polymorphisms were identified at mutual region of 3'UTR in APA2 and APA3 transcript variants. MicroRNAs (miRNAs) targeting the 3'UTRs are major posttranscriptional regulators of gene expression. Using the RegRNA2.0 (Chang et al. 2013) online prediction software, two target sites of bta-miR-2476 and bta-miR-301a in the *BBOX1* 3'UTR sequence were identified and their target sequence includes g.1797_1798insTGC and g.1935T>C loci, respectively. Interestingly, the g.1935T>C located at PAS3 resulted in "AATAAA" mutated into "AACAAA". Therefore, we speculate that these polymorphisms may be functional and directly affect the expression of the phenotype.

It is possible to localize economically important quantitative trait loci (QTL) which will expedite genetic improvement via marker-assisted selection in livestock species (Andersson and Georges 2004). In our study, although the mechanisms involved in the association of polymorphisms in the 3'UTR of *BBOX1* gene with meat quality and carcass traits are not currently well understood, a clear association with some meat quality and carcass traits was observed in the Chinese Red cattle. In the meat quality traits, cattle with the *II* and *CC* genotypes have significantly higher muscle fibre diameter than other genotypes and *NI* and *TC* genotypes have significantly lower shear force than other genotypes at the *BBOX1-Bbv*I and *BBOX1-Hin*cII loci, respectively ($P < 0.01$). In the carcass traits, cattle with the *II* and *CC* genotypes have significantly higher net meat weight and carcass weight at the *BBOX1-Bbv*I and *BBOX1-Hin*cII loci, respectively ($P < 0.01$). In the present study, the association analysis suggested that significant differences exist between the combined genotypes of *BBOX1-Bbv*I and *BBOX1-Hin*cII loci and meat quality and carcass traits ($P < 0.01$). The results are basically consistent with the results on *BBOX1-Bbv*I and *BBOX1-Hin*cII genotypes mentioned above. But a little inconsistency indicates that each of the SNPs has a small effect, and in the haplotypes the effects may combine. This coincides with the conclusion that the inheritance of haplotype combinations is more effective than that of one SNP (Stephens et al. 2001; Grindflek et al. 2004).

Our results may be a true positive test because there is some QTL evidence for meat quality and carcass traits in the respective genome region. The bovine *BBOX1* locus maps on the chromosome 15, where some QTL affecting meat and carcass traits have been previously described (McClure et al. 2012; Saatchi et al. 2013; Allais et al. 2014; Doran et al. 2014). Therefore, we can speculate that it is true that the allele itself may be functional and directly affect the expression of the phenotype, and a more likely event is that the allele is in linkage disequilibrium with another allele at a nearby locus that is the true causal allele. In addition, it is also possible that polymorphisms in the 3'UTR might have an impact on gene function by affecting the mRNA stability as well as regulatory motifs within 3'UTRs (Thomas and Saetrom 2012). Further biological and/or functional evidences are warranted to confirm the genetic effects of bovine *BBOX1* gene polymorphisms on meat quality and carcass traits.

CONCLUSION

Three different 3'UTRs APA transcript variants of bovine *BBOX1* gene were identified. Two polymorphisms, NM_001101881.2: g.1797_1798insTGC and g.1935T>C, were found in 3'UTR of *BBOX1* gene. The SNP g.1935T>C located in PAS3 sequence resulted in the shift of APA site and the presence of APA3 transcript variant. Genotypes of *BBOX1-Bbv*I and *BBOX1-Hin*cII loci were confirmed to be significantly associated with muscle fibre diameter, shear force, net meat weight, and carcass weight in cattle, respectively. We also found that combined genotypes were significantly associated with cooking loss, muscle fibre diameter, shear

https://doi.org/10.17221/105/2016-CJAS

force, net meat weight, and carcass weight. These results suggest that *BBOX1* gene may be used as a candidate for marker assisted selection in beef cattle breeding program. But further biological and/or functional evidences are needed to confirm the genetic effects of *BBOX1* gene polymorphisms on meat quality and carcass traits.

Acknowledgement. The authors thank the staff of the Branch of Animal Husbandry, Jilin Academy of Agricultural Sciences, China, for the assistance in samples collection.

REFERENCES

Allais S., Leveziel H., Payet-Duprat N., Hocquette J.F., Lepetit J., Rousset S., Denoyelle C., Bernard-Capel C., Journaux L., Bonnot A., Renand G. (2010): The two mutations, Q204X and nt821, of the myostatin gene affect carcass and meat quality in young heterozygous bulls of French beef breeds. Journal of Animal Science, 88, 446–454.

Allais S., Journaux L., Leveziel H., Payet-Duprat N., Raynaud P., Hocquette J.F., Lepetit J., Rousset S., Denoyelle C., Bernard-Capel C., Renand G. (2011): Effects of polymorphisms in the calpastatin and μ-calpain genes on meat tenderness in 3 French beef breeds. Journal of Animal Science, 89, 1–11.

Allais S., Leveziel H., Hocquette J.F., Rousset S., Denoyelle C., Journaux L., Renand G. (2014): Fine mapping of quantitative trait loci underlying sensory meat quality traits in three French beef cattle breeds. Journal of Animal Science, 92, 4329–4341.

Andersson L., Georges M. (2004): Domestic-animal genomics: deciphering the genetics of complex traits. Nature Reviews Genetics, 5, 202–212.

Barrett J.E., Fry B., Maller J., Daly M.J. (2005): HAPLOVIEW: analysis and visualization of LD and haplotype maps. Bioinformatics, 21, 263–265.

Cave M.C., Hurt R.T., Frazier T.H., Matheson P.J., Garrison R.N., McClain C.J., McClave S.A. (2008): Obesity, inflammation, and the potential application of pharmaconutrition. Nutrition in Clinical Practice, 23, 16–34.

Chang T.H., Huang H.Y., Hsu J.B., Weng S.L., Horng J.T., Huang H.D. (2013): An enhanced computational platform for investigating the roles of regulatory RNA and for identifying functional RNA motifs. BMC Bioinformatics, 14 (Suppl. 2): S4.

Doran A.G., Berry D.P., Creevey C.J. (2014): Whole genome association study identifies regions of the bovine genome and biological pathways involved in carcass trait performance in Holstein-Friesian cattle. BMC Genomics, 15, 837–848.

Grindflek E., Hoen N., Sundvold H., Rothschild M.F., Plastow G., Lien S. (2004): Investigation of a peroxisome proliferator-activated receptor gamma haplotype effect on meat quality and carcass traits in pigs. Animal Genetics, 35, 238–241.

Lopaschuk G.D., Ussher J.R., Jaswal J.S. (2010): Targeting intermediary metabolism in the hypothalamus as a mechanism to regulate appetite. Pharmacological Reviews, 62, 237–264.

McClure M.C., Ramey H.R., Rolf M.M., McKay S.D., Decker J.E., Chapple R.H., Kim J.W., Taxis T.M., Weaber R.L., Schnabel R.D., Taylor J.F. (2012): Genome-wide association analysis for quantitative trait loci influencing Warner-Bratzler shear force in five taurine cattle breeds. Animal Genetics, 43, 662–673.

Prieto N., Andres S., Giraldez F.J., Mantecon A.R., Lavin P. (2008): Ability of near infrared reflectance spectroscopy (NIRS) to estimate physical parameters of adult steers (oxen) and young cattle meat samples. Meat Science, 79, 692–699.

Rigault C., Le Borgne F., Demarquoy J. (2006): Genomic structure, alternative maturation and tissue expression of the human BBOX1 gene. Biochimica et Biophysica Acta (BBA) – Biomembranes, 1761, 1469–1481.

Rigault C., Le Borgne F., Tazir B., Benani A., Demarquoy J. (2013): A high-fat diet increases L-carnitine synthesis through a differential maturation of the BBOX1 mRNAs. Biochimica et Biophysica Acta (BBA) – Biomembranes, 1831, 370–377.

Saatchi M., Garrick D.J., Tait Jr. R.G., Mayes M.S., Drewnoski M., Schoonmaker J., Diaz C., Beitz D.C., Reecy J.M. (2013): Genome-wide association and prediction of direct genomic breeding values for composition of fatty acids in Angus beef cattle. BMC Genomics, 14, 730–744.

Stephens M., Smith N., Donnelly P. (2001): A new statistical method for haplotype reconstruction from population data. American Journal of Human Genetics, 69, 978–989.

Thomas L.F., Saetrom P. (2012): Single nucleotide polymorphisms can create alternative polyadenylation signals and affect gene expression through loss of microRNA-regulation. PLOS Computational Biology, 8, e1002621.

Tian B., Manley J.L. (2013): Alternative cleavage and polyadenylation: the long and short of it. Trends in Biochemical Sciences, 38, 312–320.

Received: 2016–11–23
Accepted after corrections: 2018–01–03