Identification of SNPs in *ME1* gene and association analysis with meat quality traits in Chinese Red cattle

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ABSTRACT: The objective of this study was to identify single nucleotide polymorphisms (SNPs) in coding regions of bovine *ME1* gene and to evaluate if the polymorphisms are associated with meat quality traits in Chinese Red cattle. Four SNPs were identified: NW_001495544:g.1721768G>A in exon 2, g.1653796T>A in exon 4, g.1649532G>A in exon 5, and g.1546272T>C in exon 12 and they were genotyped by applying the PCR-RFLP method. Statistical analysis showed that two SNPs, g.1649532G>A and g.1546272T>C, were significantly associated with cooking loss and pH_{24h} ($P < 0.05$). But no statistically significant differences were observed in the g.1721768G>A and g.1653796A>T SNPs for meat quality traits tested in Chinese Red cattle. This suggests that *ME1* gene is a candidate that may have effects on meat quality traits in cattle.

Keywords: candidate gene; polymorphism; beef; Chinese Red cattle

INTRODUCTION

Malic enzyme 1, NADP (+)-dependent, cytosolic (ME1) is a lipogenic enzyme. For *de novo* biosynthesis of fatty acids, considerable amounts of NADPH are required (Guay et al. 2007). ME1 is a ubiquitous protein responsible for the catalysis of a reversible oxidative decarboxylation reaction linking the glycolytic pathway and the citric acid cycle (Chang and Tong 2003). ME1 involved in the physiological control of energy and triglyceride synthesis is a key candidate that may have effects on meat and carcass quality (Gill et al. 2012).

Comparison of ME1 activity in Meishan and Large White pigs that differ considerably in their fatness revealed that ME1 activity is much higher in Meishan pigs (Mourot and Kouba 1999). Vidal et al. (2006) found that polymorphisms in *ME1* gene have been associated with various carcass quality traits in pigs, including backfat thickness and muscular pH. A SNP was detected in 3' UTR of *ME1* gene in Chinese Red cattle and this mutation creates a *Dra*I restriction site. The *ME1-Dra*I polymorphism was shown to be associated with meat quality and carcass traits in Chinese red cattle (Zhou et al. 2011). Gill et al. (2012) have demonstrated that the c.903 + 11A>T and c.1001 + 66C>T SNPs in bovine *ME1* gene are associated with taste panel assessed juiciness and pH at 24 h, respectively.

Chinese Red cattle is an indigenous breed in China that was established in 1985. It has strong tolerance to cold or hot climate, strong resistance to disease, tender and delicious meat (Li et al. 2010). The main objective of this study was to investigate polymorphisms in the coding regions of the bovine *ME1* gene and to analyze the relationship between polymorphisms and meat quality traits of Chinese Red cattle.

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

Supported by the Platform of Germplasm Resources of Domestic Animals, Natural Science Fund of Shandong Province of the P.R. China (Project No. ZR2012CL19) and by the Liaocheng University Start-up Fund for Doctoral Scientific Research.

conducted, and also approved and authorized by the Chinese Ministry of Agriculture.

Animals and genomic DNA isolation. Blood samples and phenotypic data on meat traits were obtained from 344 Chinese Red steers reared at two experimental stations of the Branch of Animal Husbandry, Jilin Academy of Agricultural Sciences, China from 2007 to 2011. They were the progenies of 9 sires and unrelated dams (18–46 progenies per sire). Their slaughter ages ranged from approximately 24 to 26 months. Genomic DNA was isolated from blood samples and stored at –20°C. Meat quality traits were measured according to the criteria GB/T17238-2008 Cutting Standard of Fresh and Chilled Beef in China (China Standard Publishing House), and also according to reference methods described by Pannier et al. (2009) and Allais et al. (2010, 2011).

Polymorphism identification and genotyping. Fourteen primer pairs (**Table S1**) were designed based on NCBI database (GenBank accession Nos. NW_001495544.2 and FJ515746.1) to amplify exons and parts of their flank introns in *ME1* gene using OLIGO Primer Analysis software (Version 6.0, 2000). Thirty Chinese Red steers were randomly chosen for screening DNA polymorphisms using polymerase chain reaction – single strand conformation polymorphism (PCR-SSCP) method. The PCR products of different electrophoresis patterns were purified with Axygen kits (MBI Fermentas, Vilnius, Lithuania) and were sent to Sangon Biotech (Shanghai) Co., Ltd. for sequencing in both directions in an ABI PRIZM 377 DNA sequencer (Perkin-Elmer, Foster City, USA). The sequences were analyzed with BioXM software (Version 2.6, 2004).

All polymorphisms identified by PCR-SSCP and sequencing were confirmed by PCR restriction fragment length polymorphism (RFLP) method using relevant restriction enzymes (MBI Fermentas) (Table 1). The SNPs identified in exons were genotyped by PCR-RFLP method in a total of 344 Chinese Red steers. The resulting DNA fragments

Table 1. Identified SNPs in *ME1* gene of Chinese Red cattle

Location	Sequence*	Polymorphism position (Accession No. NW_001495544.2)	Predicted amino acid changes	Restriction enzyme
Intron 1	GGCTC(A/G)ATCTC	g.1722876A>G	not applicable	*Taq*I
	AATCT(G/C)ATCAA	g.1722871 G>C		*Bcl*I
	CCCTC(A/G)AATTA	g.1721890A>G		*Taq*I
Exon 2	TTCGA(G/A)TACTT	g.1721768 G>A	missense (Val/Ile)	*Rsa*I
Intron 2	AATGG(G/A)ACATA	g.1713203 G>A	not applicable	*Bsm*FI
Exon 4	TGGCC(T/A)GAAGA	g.1653796 T>A	synonymous	*Bal*I
Intron 4	AATGT(A/G)CCACA	g.1653748 A>G	not applicable	*Rsa*I
	CTACC(GC/AT)TACCT	g.1653690_1653691delinsAT		*Aci*I
	TATAA(G/-)TTGAA	g.1653666delG		*Tas*I
	TCAAT(C/T)GGATA	g.1653657C>T		*Mun*I
Exon 5	CAACA(G/A)TGTTT	g.1649532 G>A	synonymous	*Taa*I
Intron 5	TATAG(C/T)TGTTA	g.1649469 C>T	not applicable	*Alu*I
	TACTT(T/C)TTAAG	g.1649455 T>C		*Afl*II
Intron 7	GATCC(A/G)TATGC	g.1571840 A>G	not applicable	*Nde*I
	CCTTA(T/G)AAAAT	g.1571803 T>G		*Dde*I
	ATAGG(TG/CA)TCTAG	g.1571766_1571767delinsCA		*Sfa*NI
	TTCAT(A/G)GGTGT	g.1571763A>G		*Hin*1II
	AAACA(C/A)AATTC	g.1571745 C>A		*Apo*I
	CAGAG(-/AAGCTT)TCTCA	g.1571694_1571695insAAGCTT		*Hin*dIII
	AGTAC(T/A)TTCTA	g.1571680 T>A		*Sca*I
Intron 8	TACCT(C/G)CCTTA	g.1560874 C>G	not applicable	*Bsp*MI
	TAACT(C/T)ATGAA	g.1560815 C>T		*Hin*1II
Intron 11	AAAGC(T/C)TGATC	g.1547395 T>C	not applicable	*Hin*dIII
Exon 12	AACAA(T/C)TCCTA	g.1546272 T>C	synonymous	*Tas*I

*bases in brackets show that the mutants create/disrupt a restriction site for corresponding restriction enzyme

were separated by gel electrophoresis in 1–3% (w/v) agarose in 1×Tris-acetate-EDTA buffer and were stained with ethidium bromide.

Statistical analyses

Genotypic and allelic frequencies were calculated using the POPGENE software (Version 1.31, 1999). Single marker association analysis was carried out to evaluate the relationships between genotypes of each exon SNP and meat traits in Chinese Red cattle population using PROC GLM procedure of SAS (Statistical Analysis System, 1999). The phenotypic data included cooking loss, muscle fibre diameter, shear force, drip loss, and pH_{24h}. 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 i^{th} genotype
S_j = effect of j^{th} sire
YS_k = effect of k^{th} season of slaughter
b = regression coefficient for slaughter age in days
D_l = slaughter age in days
e_{ijkl} = random residual error

RESULTS

Polymorphisms of the bovine ME1 gene. A total of 24 polymorphisms were discovered in the examined regions of the *ME1* gene. Among these polymorphisms, twenty-one were SNPs, two were deletion/insertion mutations (g.1653690_1653691delinsAT and g.1571766_1571767delinsCA), and one was insertion mutation (g.1571694_1571695insAAGCTT). Out of 21 SNPs, four were in the coding regions (exons), and the others were intronic. However, only the g.1721768G>A SNP was a nonsynonymous mutation that replaced amino acid valine to isoleucine in the polypeptide chain. Details of all polymorphisms, mutation types, and their flanking sequences are presented in Table 1.

Genotyping was performed for the four SNPs in coding regions by the PCR-RFLP method. Genotype and allelic frequencies of the four SNPs are listed in Table 2. The DNA restriction fragments for the RFLPs: *ME1-Rsa*I, *ME1-Bal*I, *ME1-Taa*I, and *ME1-Tas*I are also shown in Table 2.

Association analysis. Four SNPs in coding regions of *ME1* gene were genotyped in order to study the possible association with meat quality trait values. The evaluation of meat quality traits in Chinese Red steers based on genotypes is shown in Table 3. The *ME1-Taa*I (g.1649532G>A) genotypes revealed a significant association with cooking loss and pH_{24h} ($P < 0.05$). The cattle with the *TT* genotype had a significantly lower cooking loss and higher pH_{24h} compared to animals with the *CC* genotype. The *ME1-Tas*I (g.1546272T>C) genotypes revealed a significant association with cooking loss and pH_{24h} ($P < 0.05$). The cattle with the *TT* genotype had a significantly lower cooking loss and higher pH_{24h} compared to animals with the *TC* genotype. In *ME1-Rsa*I (g.1721768G>A) and *ME1-Bal*I (g.1653796A>T) loci no statistically

Table 2. Genotypic and allelic frequencies for four single nucleotide polymorphisms (SNP) loci in coding region of *ME1* gene

SNP	Genotype (*n* given in brackets)	Genotype frequency	Allele (restriction fragments size)	Allele frequency
*ME1-Rsa*I (g.1721768 G>A)	*GG* (264)	0.77	*G* (143 and 64 bp)	0.81
	GA (28)	0.08	*A* (207 bp)	0.19
	AA (52)	0.15	–	–
*ME1-Bal*I (g.1653796 A>T)	*AA* (224)	0.65	*A* (270 bp)	0.77
	TA (79)	0.23	*T* (211 and 59 bp)	0.23
	TT (41)	0.12	–	–
*ME1-Taa*I (g.1649532G>A)	*GG* (251)	0.73	*G* (174 and 121 bp)	0.78
	GA (38)	0.11	*A* (295 bp)	0.22
	AA (55)	0.16	–	–
*ME1-Tas*I (g.1546272T>C)	*TT* (272)	0.79	*T* (275 and 50 bp)	0.90
	TC (72)	0.21	*C* (325 bp)	0.10

Table 3. Least Squares Means and standard error of the GLM models of the different *ME1* genotypes for the meat quality traits tested

Loci	Genotype	Meat quality trait (LSM ± SD)				
		cooking loss (%)	muscle fibre diameter (μm)	shear force (kg)	drip loss (%)	pH_{24h}
*ME1-Rsa*I (g.1721768 G>A)	*GG*	40.29 ± 2.37	34.30 ± 3.02	3.78 ± 0.36	2.35 ± 0.69	5.54 ± 0.11
	GA	40.01 ± 2.45	33.59 ± 1.99	3.81 ± 0.31	2.25 ± 0.62	5.56 ± 0.09
	AA	39.75 ± 2.24	33.89 ± 3.21	3.82 ± 0.31	2.15 ± 0.60	5.54 ± 0.10
*ME1-Bal*I (g.1653796 A>T)	*AA*	40.12 ± 2.32	34.21 ± 3.15	3.84 ± 0.34	2.24 ± 0.62	5.54 ± 0.10
	AT	40.23 ± 2.57	34.13 ± 2.94	3.83 ± 0.30	2.37 ± 0.73	5.55 ± 0.10
	TT	40.31 ± 1.98	34.25 ± 2.48	3.77 ± 0.40	2.44 ± 0.67	5.52 ± 0.12
*ME1-Taa*I (g.1649532 G>A)	*GG*	40.40 ± 2.27^{a}	34.19 ± 2.99	3.80 ± 0.35	2.29 ± 0.67	5.53 ± 0.10^{a}
	GA	40.04 ± 2.07^{ab}	33.68 ± 2.36	3.72 ± 0.45	2.45 ± 0.77	5.54 ± 0.13^{ab}
	AA	39.37 ± 2.74^{b}	34.50 ± 3.30	3.78 ± 0.27	2.32 ± 0.60	5.57 ± 0.11^{b}
*ME1-Tas*I (g.1546272 T>C)	*TT*	39.47 ± 2.74^{a}	33.80 ± 2.35	3.85 ± 0.22	2.36 ± 0.62	5.58 ± 0.11^{a}
	TC	40.35 ± 2.23^{b}	34.29 ± 3.12	3.77 ± 0.37	2.30 ± 0.68	5.53 ± 0.10^{b}

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

^{a,b}means with different superscripts are significantly different ($P < 0.05$)

significant differences were observed for meat quality traits tested.

DISCUSSION

In the present study, we have identified 24 polymorphisms of *ME1* gene in exons and their flanking introns. Gill et al. (2012) have identified six polymorphisms in introns 4, 9, 10 and exon 8 of *ME1* gene in Aberdeen Angus beef cattle. But these polymorphisms were not detected in Chinese Red cattle in our research. Therefore, we deduce that it is due to a difference between breeds.

Polymorphisms of *ME1* gene have been shown to influence meat quality and carcass traits in beef cattle (Zhou et al. 2011; Gill et al. 2012). Gill et al. (2012) revealed an association between the c.1001+66C>T SNP in *ME1* gene and pH_{24h} in Aberdeen Angus beef cattle population. The *T* allele at the c.903+11A>T SNP was associated with an increase in taste panel assessed juiciness. Here we also found an association of the *ME1-Taa*I and the *ME1-Tas*I loci with cooking loss and pH_{24h} in Chinese Red cattle population. Clearly these loci associated with meat quality in Chinese Red cattle are different from those previously reported in other breeds. Though the silent SNPs in *ME1* gene – g.1649532G>A and g.1546272T>C SNPs – do not alter the encoded amino acid, they may affect the splicing of the transcript, mRNA stability, and protein functions, as it was shown in the case of other genes (Capon et al. 2004; Nackley et al. 2006; Kimchi-Sarfaty et al. 2007; Rhodri and Deane 2010). However, further studies are required to detect possible effects of these SNPs on expression level of *ME1* gene.

We also observed that animals with the *T* allele at the *ME1-Taa*I locus had a higher pH_{24h} and a lower cooking loss. The cattle with the *TT* genotype at the *ME1-Tas*I locus had a lower cooking loss and a higher pH_{24h}. The change trends of pH_{24h} and cooking loss in our research results are similar with results presented in Hampshire pigs by Monin and Sellier (1985). A similar effect of *ME1* genotypes on pH was also reported in pigs (Vidal et al. 2006). Muscle pH is influenced by the production of lactate through the anaerobic glycogen metabolism (Pearson and Young 1989). Thus, this association is relevant because pyruvate, which is synthesized in the reaction catalyzed by ME1, can be converted to lactate by the lactate dehydrogenase enzyme. In addition, the bovine *ME1* locus maps on the chromosome 9, where a quantitative trait locus (QTL) affecting retail product yield and fat deposition have been reported (Casas et al. 2003). It is also likely that the silent g.1649532G>A and g.1546272T>C may be linked to the nearby

QTL, or that causative mutations in other regions of the *ME1* gene are responsible for these phenotypic alterations. Considering the economic importance of the meat quality traits to the livestock industry, further investigations on the bovine *ME1* gene are clearly essential.

Acknowledgement. The authors thank the workers of the Branch of Animal Husbandry for their assistance in sample collection. We thank Zhaozhi LI and Jin LI for help in genotyping.

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Received: 2013–04–08
Accepted after corrections: 2014–02–18

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