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Full Length Research Paper

Association of *MC4R* gene variants with carcass and meat quality traits in Qinchuan cattle

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MC4R **belongs to a seven-transmembrane G-protein-coupled receptor which may regulate body composition and insulin action. Many mutations in the *MC4R* gene are associated with obesity, energy expenditure and serum triglyceride levels in human and animals. Six mutations in the *MC4R* gene were identified in our study (-293 C>G, -193 A>T, -192 T>G, -129A>G, -84T>C and 1069C>G). The -129A>G was significantly associated with live weight (LW) (P<0.05); cattle with the genotypes AG and GG had higher LW than genotype AA. The 1069 C>G was significantly associated with live weight (LW), carcass weight (CW), back fat thickness (BFT) and marbling score (MS). Cattle with the genotype GG had higher LW, CW and MS than genotype CC; cattle with the genotypes GG and CG had higher MS than CC. The results suggested that -129 A>G and 1069 C>G SNP of the *MC4R* gene may be useful as a genetic marker for carcass and meat quality traits in Qinchuan cattle.**

Key words: *MC4R* gene, polymorphism, meat quality, carcass traits, qinchuan cattle.

INTRODUCTION

The melanocortin-4 receptor (*MC4R*) belongs to a seven-transmembrane G-protein-coupled receptor expressed in the brain, predominantly in the hypothalamus. MC4R signaling is an important mediator of leptin's effects on food intake and body weight (Seeley et al., 1997) and it signals through activation of adenylate cyclase. Activation of the *MC4R* gene results in the inhibition of food intake, a targeted disruption of the *MC4R* gene in mice causes obesity associated with hyperphagia, hyperinsulinemia and hyperglycinemia (Huszar et al., 1997; Ste Marie et al., 2000). In addition, the *MC4R* gene may regulate body composition and insulin action because *MC4R* agonists given to rats intracerebrially or humans intranasally decrease visceral fat and increase insulin sensitivity (Fehm et al., 2001; Obici et al., 2001). Some studies reported that many mutations in the *MC4R* gene are associated with human obesity, energy expenditure and serum triglyceride levels (Bronner et al., 2006; Dempfle et al., 2004; Zobel et al., 2009). A missense mutation (Asp298As n) has been described in the *MC4R* locus in pigs (Kim et al., Heid et al., 2008; Kobayashi et al., 2002; Rutanen et al., 2004; Sina et al., 1999; Vaisse et al., 2000; Yeo et al., 1998; 2000), and the mutation has been associated with daily food intake, growth, fat deposition, lipid composition of muscle and fat tissues (Hernández-Sánchez et al., 2003; Jokubka et al., 2006; Kim et al., 2000; Ovilo et al., 2005). These indicate that the *MC4R* gene is an excellent candidate gene for feeding, fatness or growth-related traits in livestock.

Bovine *MC4R* gene has been isolated and mapped to BTA 24q27 by radiation hybrid mapping. Several SNPs in the coding region of bovine *MC4R* gene have been detected by PCR–SSCP or PCR–RFLP (Haegeman et al., 2001; Thue et al., 2001; Valle et al., 2004). Moreover, SNPs of the *MC4R* genes associated with growth traits in cattle were detected (Zhang et al., 2006; Zhang et al., 2008). In our study, SNPs in the bovine *MC4R* gene were investigated, and their associations with cattle carcass

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Table 1. The primer sequences and their information of bovine *MC4R* gene.

Primer	The primer sequences	Size (bp)	Tm (℃)	Location
MC4R1	F: 5' AGCAGCAGCGTCTGACACTC 3' R: 5' ATGGTTTCCGACCCGTTG 3'	735	51.4	5'-untranslated region and exon
MC4R2	F: 5' TGACTCGGTGATCTGTAGC 3' R: 5' TTCACTCCATGCCCTACA 3'	741	58.9	Exon
MC4R3	F: 5' TTGACCCTCTGATTTATGCC 3' R: 5' AATCCCAAATTGCCTGTGAG 3'	812	59.8	exon and 3'- flanking

and meat quality traits were analyzed.

MATERIALS AND METHODS

Animals and data collection

A total of 787 animals including Qinchuan (n=422), Luxi (n = 77), Nanyang (n=40), Xianan (n=86), Jiaxian Red (n=71), Angus (n=55), Anxi (n=36) were randomly selected from commercial populations and used to analyze the MC4R allelic frequencies, which were reared in the province of Shaanxi, Shandong, Henan and Shanxi respectively. A total of 101 of Qinchuan were used for the association study. Carcass and meat quality traits were measured according to the criterion of cutting standard of fresh and chilled beef in China (GB/T 17238-1998). The traits were measured or calculated as follows: live weight (LW), carcass weight (CW), dressing percentage (DP), carcass length(CL), carcass chest depth (CCD), hind leg length (HLL), backfat thickness (BFT), logissimus muscle area (LMA), marbling score (MS), water holding capacity (WHC), and meat tenderness (MT). DNA samples were extracted from leukocytes and tissue samples according to Mullenbach et al. (1989).

SNP identification and genotyping

According to the sequence of bovine the *MC4R* gene (GenBank accession No. NW_001494269.1), three pairs of primers were designed to amplify the bovine *MC4R* gene (Table 1). Polymerase chain reaction (PCR) amplifications were performed in 20 µl volume containing 50 ng DNA template, 10 pM of each primer, 0.20 mM dNTP, 2.5 mM $MgCl_2$, and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). The PCR condition was 94℃ for 5 min followed by 35 cycles of 94℃ for 30 s, annealing for 30 s, and 72℃ for 30 s and a final extension at 72℃ for 10 min.

Single-strand conformation polymorphism (SSCP) method was used to scan mutations within the amplified regions. Aliquots of 10 µl PCR products were mixed with 10 µl denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene cyanole and 0.025% bromophenol blue), heated for 10 min at 98℃ and chilled on ice for 5 min. Denatured DNA was subjected to 10% PAGE (Polyacrylamide Gel) in 1 × TBE buffer and constant voltage (200 V) for 2.5 - 3.0 h at a constant temperature of 12℃, then gels were stained with 0.1% silver nitrate (Sun et al., 2002).

The PCR products which represented different PCR-SSCP genotypes, including both homozygous and heterozygous genotypes were purified with the AxyPrep PCR DNA Purification Kit (Axygen, China) and sequenced using the ABI 377 sequencer from both directions (Applied Biosystems, USA).

Sequences were aligned using web based on CLUSTAL-W (http://www.ebi.ac.uk/clustalw/index.html) program.

The -293C>G, -129A>G and 1069C>G SNPs could be genotyped by restriction enzymes TaiI. Aliquots of 15 µl PCR products were digested with 15 units endonuclease (MBI, Fermentas) at 37℃ for 5 h following the supplier's directions. The restriction fragments were scored and analyzed by electrophoresis on 3% agarose gels.

Statistical analysis

The data were expressed as mean ± standard deviation (SD). Gene frequencies were determined by direct counting. Statistical analysis of associations was performed between *MC4R* genotypes and carcass and meat quality traits of Qinchuan steers using SPSS (version 13.0). The model applied was:

$$Y_{ijk}=\mu+A_j+M_k+e_{ijk}$$

Y_{ijk} was stands for observed value; µ wasoverall mean for each trait; A_j was the fixed effect of j_{th} age of slaughtering; M_k was the fixed k_{th} genotype marker; e_{ijk} = random error.

RESULTS AND DISCUSSION

SNP marker genotyping

A 735, 741 and 812 bp fragments of the *MC4R* gene were amplified and sequenced. Six mutations were identified. The -293 C>G, -193 A>T, -192 T>G, -129A>G and -84T>C mutations were detected in 5'-untranlated region; the 1069C>G mutation was detected in exon (Figure 1). Previous studies have shown that seven SNPs have been detected in the MC4R gene of *Bos taurus* (Haegeman et al., 2001; Thue et al., 2001; Valle et al., 2004; Zhang et al., 2006). 927C>T mutation were not found in our study.

Fortunately, the mutations of -293C>G and -129A>G linkage result in two TaiI restriction site. The 1069C>G mutation caused amino acid mutation Val to Leu, which created a TaiI restriction site. In the analyzed population, for the -293C>G and -129A>G linkage mutation, four size variants of restriction fragments were identified, namely; 735, 137, 164 and 434 bp. An analysis of the localization of migration bands of the restriction fragments enabled to identify three genotypes of mutation C>G and A>G. The genotype AA represents the occurrence of one band of 735 bp, genotype AG represents four restriction fragment bands of 735, 434, 164 and 137 bp and genotype GG represents three bands of 434, 164 and 137 bp. For the 1069C>G SNP, three size variants of restriction fragments were identified, namely: 741, 481 and 260 bp. The

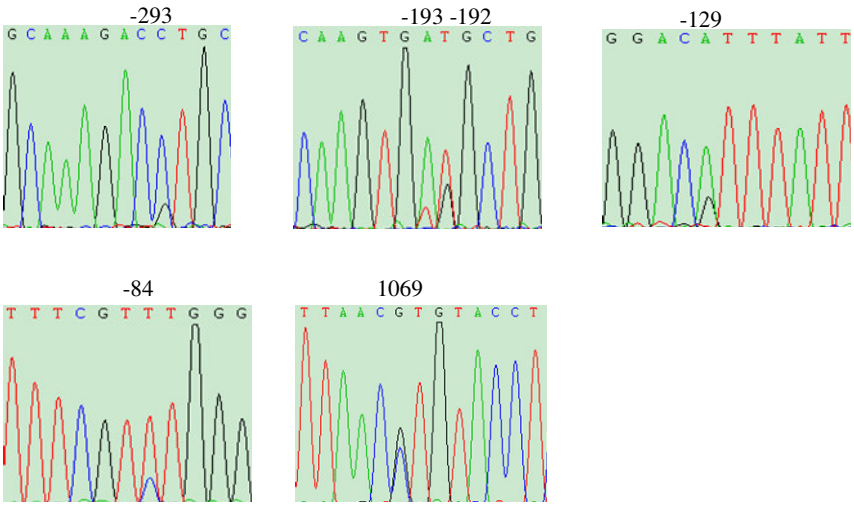

Figure 1. Partial sequencing maps of heterozygotes discriminated by PCR–SSCP in bovine MC4R gene.

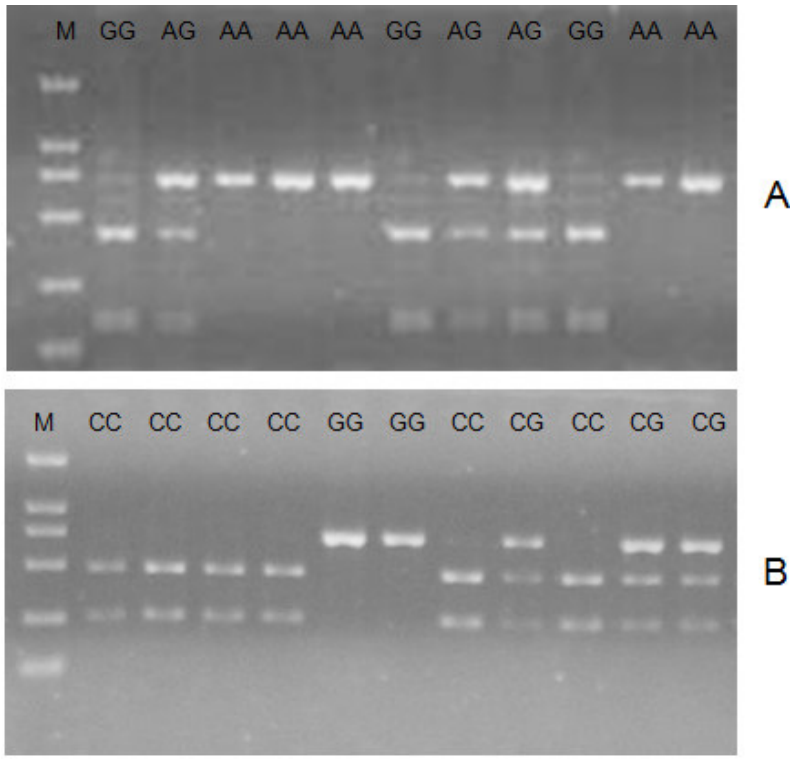

Figure 2. Agrose gel (3.0%) showing different genotypes of bovine MC4R gene. M: 100 - 2000 bp. A and B represent the -129A>G and 1069C>G loci respectively. The genotypes are given at the top of the columns.

genotype GG represents the occurrence of one band of 741 bp, genotype CG represents three restriction fragment bands of 741 bp, 481 bp and 260 bp, and genotype GG represents two bands of 481 bp and 260 bp. The electrophoresis of the PCR products is shown in Figure 2. Allele frequencies of the two SNPs were investigated in seven different beef populations (Table 2). Frequencies of -129A and 1069C were ranged from 0.54 to 0.99 and 0.29 to 0.77 in seven breeds of cattle, respectively.

SNP marker associations

The genotypes of 101 individuals were compared with

Table 2. Genotype frequencies and allelic frequencies of MC4R gene determined by PCR–RFLP in the seven populations.

Breed	-129A>G					1069C>G				
	Genotype frequencies			Allele frequencies		Genotype frequencies			Allele frequencies	
	AA	AG	GG	A	G	CC	CG	GG	C	G
Qinchuan	0.29	0.51	0.20	0.55	0.45	0.28	0.54	0.18	0.55	0.45
Luxi	0.31	0.46	0.23	0.54	0.46	0.21	0.35	0.44	0.38	0.62
Nanyang	0.50	0.45	0.05	0.73	0.27	0.03	0.52	0.45	0.29	0.71
Xianan	0.45	0.36	0.19	0.63	0.37	0.16	0.41	0.43	0.37	0.63
Jiaxian Red	0.45	0.46	0.09	0.68	0.32	0.21	0.41	0.38	0.42	0.58
Angus	0.64	0.27	0.09	0.77	0.23	0.13	0.40	0.47	0.33	0.67
Anxi	0.97	0.03	0.00	0.99	0.01	0.65	0.25	0.10	0.77	0.23

Table 3. Associations of -129A>G SNP genotypes with carcass and meat quality traits at bovine MC4R gene.

Trait	Genotypes of Tail PCR-RFLP genotyping			p-value
	AA (mean ± SD)	AG (mean ± SD)	GG (mean ± SD)	
Live weight (LW)/kg	489.25a ± 7.59	505.90a ± 7.53	507.20b ± 7.33	0.048*
Carcass weight (CW)/kg	264.90 ± 5.74	267.48 ± 8.31	269.28 ± 6.44	0.686
Dressing percentage (DP)/%	0.54 ± 0.01	0.53 ± 0.02	0.53 ± 0.01	0.569
Carcass length (CL)/cm	148.50 ± 1.91	149.45 ± 2.03	149.80 ± 2.05	0.620
Carcass chest depth (CCD)/cm	26.75 ± 3.40	25.08 ± 2.99	25.60 ± 2.61	0.647
Hind leg length (HLL)/cm	71.50 ± 1.91	71.50 ± 2.17	71.80 ± 2.39	0.966
Backfat thickness (BFT)/cm	0.89 ± 0.09	0.95 ± 0.11	0.96 ± 0.14	0.376
Loin muscle area (LMA)/cm2	84.96 ± 8.43	85.97 ± 8.00	88.82 ± 9.29	0.528
Marbling score (MS)/1-5	2.22 ± 0.97	2.13 ± 0.85	2.17 ± 0.72	0.956
Water holding capacity (WHC)/%	0.22 ± 0.03	0.22 ± 0.05	0.21 ± 0.04	0.846
Meat tenderness (MT)/kg	4.25 ± 0.55	4.12 ± 0.48	4.18 ± 0.57	0.816

Data with a different letters (a, b) within the same line are different significantly at $P < 0.05$, respectively. *Effect was significant at $P < 0.05$.

Table 4. Associations of 1069C>G SNP genotypes with carcass and meat quality traits at bovine MC4R gene

Trait	Genotypes of Tail PCR-RFLP genotyping			p-value
	CC (mean ± SD)	CG (mean ± SD)	GG (mean ± SD)	
Live weight (LW)/kg	490.25^{a} ± 12.12	505.90^{ab} ± 14.53	512.80^{b} ± 10.71	0.044*
Carcass weight (CW)/kg	256.10^{a} ± 12.86	270.48^{ab} ± 17.67	281.52^{b} ± 8.20	0.047*
Dressing percentage (DP)/%	0.52 ± 0.02	0.53 ± 0.03	0.55 ± 0.02	0.217
Carcass length (CL)/cm	148.25 ± 3.30	149.45 ± 2.03	149.20 ± 2.28	0.700
Carcass chest depth (CCD)/cm	24.50 ± 2.38	25.08 ± 2.99	24.40 ± 2.07	0.875
Hind leg length (HLL)/cm	71.25 ± 2.63	71.50 ± 2.17	71.80 ± 2.39	0.938
Backfat thickness (BFT)/cm	0.82^{a} ± 0.11	0.95^{b} ± 0.11	0.96^{b} ± 0.13	0.039*
Loin muscle area (LMA)/cm2	79.06 ± 10.81	85.97 ± 8.00	89.37 ± 8.63	0.073
Marbling score (MS)/1-5	2.60^{a} ± 0.45	2.13^{ab} ± 0.85	2.13^{b} ± 0.64	0.038*
Water holding capacity (WHC)/%	0.22 ± 0.02	0.22 ± 0.05	0.22 ± 0.05	0.852
Meat tenderness (MT)/kg	4.17 ± 0.37	4.12 ± 0.48	4.16 ± 0.54	0.961

Data with a different letters (a, b) within the same line are different significantly at $P < 0.05$, respectively. * Effect was significant at $P < 0.05$.

MS ($P < 0.05$) (Table 4) at the SNP maker of 1069C>G in exon. Cattle with the genotype GG had higher LW, CW and MS than genotype CC; there was no significant difference between CG and CC or GG. Cattle with the genotypes GG and CG had higher MS than CC. Cattle with the genotype GG was higher than CG ($P > 0.05$) and CC ($P > 0.05$) in LMA. These results suggested that -129G and 1069G may contribute to the increment of LW in cattle; 1069G may increase CW, BFT and MS.

Research on mutations in targeted functional genes (candidate genes) and their association with economic traits is performed to ascertain the genetic basis of production traits and to develop DNA tests as selection tools in animal breeding schemes (De Vries et al., 1998). Many of these discoveries were reported and used in combination with performance information to improve animal production (Gan et al., 2008; Jokubka et al., 2006). The present study showed that the -129A>G was significantly associated with live weight; 1069C>G was significantly associated with live weight, carcass weight, backfat thickness, marbling score. These results were similar to the reports of Zhang et al. (2008) that a significant associated with birth weight and average daily gain in Nanyang cattle.

In conclusion, we identified SNPs in the *MC4R* gene and investigated their association in several populations. Our results provided evidence that the *MC4R* gene might have potential effects of carcass and meat quality traits. In the further works, we need to use the SNPs for marker-assisted selection in larger population and investigate whether the mutations of *MC4R* gene play a role in those traits.

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