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Full Length Research Paper

Identification of SNPs in chemerin gene and association with carcass and meat quality traits of Qinchuan Cattle

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Chemerin is a novel adipokine that regulates adipogenesis and adipocyte metabolism via its own receptor. In this study, two novel SNPs (868A>G in exon 2 and 2692C>T in exon 5) of chemerin gene were identified by PCR-SSCP and DNA sequencing technology. The allele frequencies of the novel SNPs were determined in the genetically diverse bovine breeds including six Chinese indigenous cattle breeds (Caoyuan red, Jiaxian red, Luxi, Nanyang, Qinchuan and Xia'nan cattle). We evaluated the potential association of the SNPs with traits measured by ultrasound measurement in 214 Qinchuan individuals. Furthermore, meat quality traits data gotten from carcass measurement in another 69 Qinchuan individuals were analyzed by the comparison between the genotypes and their phenotypic data. Results showed that SNP 868A>G had a significant association with the ultrasound loin-muscle area (P < 0.05), loin-eye area and water holding capability (P < 0.05). And also revealed significant effects of genotype on the ultrasound backfat thickness (P < 0.05), backfat thickness and water holding capability (P < 0.05) of SNP 2692C>T. It was shown that associations do exist between chemerin gene and carcass and meat quality traits. As a result of the small sample size of this study, it is proposed that further effort is required to validate these findings in larger populations. It could be concluded that ultrasound measurements are similar in accuracy to carcass measurements for predicting carcass and meat quality traits in cattle, and could be a useful predictor of retail yield in live animals.

Key words: Bos bovine, chemerin gene, PCR-SSCP, SNP, meat quality traits.

INTRODUCTION

Chemerin is a new adipokine associated with obesity and the metabolic syndrome in human (Bozaoglu et al., 2007) and mouse (Ernst et al., 2010). Chemerin gene is also known as retinoic acid receptor responder 2 (RARRES2) and tazarotene-induced gene 2 (TIG2) (Nagpal et al., 1997), has been isolated as a novel chemoattractive agonistic protein binding to the G-protein-coupled receptor ChemR23 (Gantz et al., 1996; Meder et al., 2003), also known as chemokine-like receptor-1 (CMKLR1) (Wittamer et al., 2003; Zabel et al., 2005). The chemerin gene of Bos bovine is located on chromosome 4 (GenBank: NC_007302) and consists of six exons, with exon 2, 3, 4 and exon 5 coding a protein with 162 amino acid (Song et al., 2010). Through its binding to chemerinR, chemerin is involved in regulating adipogenesis and adipocyte metabolism (Goralski et al., 2007), innate and adaptive immunity (Meder et al., 2003; Wittamer et al., 2005; Zabel et al., 2005), bone development (Methner et al., 1997) and immunodeficiency virus infections (Martensson et al., 2006). It potentiates insulin-stimulated glucose uptake and insulin signaling in 3T3-L1 adipocytes, which identifies chemerin as a novel adipokine (Takahashi et al., 2008). It was reported that chemerin gene was expressed in many tissues, such as liver, lung, pituitary glands, ovaries, kidney and so on (Bozaoglu et al., 2007; Roh et al., 2007), but white adipose tissue was the only histiocyte that express high level chemerin and ChemerinR (Goralski et al., 2007; Meder et al., 2003).

Recently, Song et al. (2010) cloned chemerin gene and acquired its receptor gene from the adipose tissues of

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Japanese Black cattle, and found that their DNA sequences and amino acid sequence were highly homologous to those of humans, mice and pigs, and the bovine chemerin mRNA was highly expressed in the adipose and liver tissues than the other histiocyte. In addition, they found that the transcripts of chemerin and expression of its receptor were up-regulated during adipocyte differentiation. So, chemerin primarily acts on adipogenesis and adipocyte metabolism through its own receptor. To our knowledge, few polymorphisms of bovine chemerin gene have been reported. Based on the important roles in organisms, it could be a potential gene for carcass and meat quality traits in bovine. Therefore, the objective of this study was to detect SNPs of chemerin gene in bovine and explore their possible association with carcass and meat quality traits in Qinchuan cattle breed.

MATERIALS AND METHODS

Sample collection and DNA extraction

Individuals were randomly selected from six cattle breeds of 627 adult animals as follows: Qinchuan (QC, no = 214, Shaanxi province of China), Caoyuan red (CYR, no = 112, Jilin province of China), Jiaxian red (JR, no = 71, Henan province of China), Luxi (LX, no = 69, Shandong province of China), Nan yang (NY, no = 81, Henan province of China) and Xia'nan cattle (XN, no = 76, Henan province of China). Ultrasound measurements were available for 214 Qinchuan cattle (Brethour et al., 1994; Hamlin et al., 1995) including ultrasound backfat thickness (UBF), ultrasound loin-muscle area (ULMA) and ultrasound marbling score (UMAR). As we know, ultrasound technology has been used extensively used in beef cattle and swine enterprises over the past decade, and its use is continuing to gain popularity (Wall et al., 2004). Apart from that 69 individual Qinchuan heifers 2.5 to 3.0 years old were selected randomly and slaughtered in Shaanxi Kingbull Livestock Development Co., Ltd, and data with seven meat quality traits were collected, including live weight (LW), carcass weight (CW), backfat thickness (BFT), loin-eye area (LEA), marble score (MAR), water holding capacity (WHC) and tenderness (TD).

DNA preparation

The animal's blood samples were obtained and treated with 2% heparin and stored at -80°C. And the genomic DNA was extracted from blood leucocytes by a standard phenol-chloroform protocol method (Mullenbach et al., 1989).

PCR conditions

In order to amplify DNA region, primer A (F: 5'-CAGGAGACGGAGGTGAAGC-3', R:5'-CACCGTGTCTGCCGCATT-3';) and primer B (F:5'-GTGGTAGGCGCTGGCAGGAA-3'; R:5'-CGTGAGGGAGGCGGTCTTT-3') were designed to amplify 196 and 288 bp fragments from exon 2 and exon 5 of the bovine chemerin gene (GenBank: NC_007302) by Primer 5.0 software, respectively. Each PCR was performed in a 20-µl reaction volume containing 50 ng genomic DNA, 10 mM of each primer, 2.5 mM $Mgcl_2$, 0.20 mM dNTP and 0.5U Taq DNA polymerase (TaKaLa, Dalian, China). The cycling protocol was 5 min at 95°C, 32 cycles of 94°C for 30 s, 58.3°C (or 64.5°C) annealing for 30 s, 72°C for 35 s, with a final extension at 72°C for 1 0 min. PCR products were electrophoresis on 1.5% agarose gels. Then the products for sequencing were purified with Axygen kits (MBI Fermentas, Canada) and sequenced in both directions in an ABI PRIZM 377 DNA sequencer (Perkin-Elmer, USA). The sequences were analyzed with the SeqMan software.

SSCP polymorphism and sequencing

Aliquots of 6 µl of the 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°C and chilled on ice immediately after heated. Then 16 µl of this mixture was applied to a 12% polyacrylamide gel (29:1 acrylamide:bis), 14% (V/V) glycerol and 10 × TBE buffer, Electrophoresis was carried out with 1×TBE buffer at 250 V for 30 min and 115 V for 14 h at room temperature. The gel was stained with 0.1% silver nitrate (Lan et al., 2007) and visualized with 2% NaOH solution (containing 0.1% formaldehyde) according to Zhang et al. (2007). After the polymorphism was detected, the PCR products of different electrophoresis patterns were sequenced in both directions in an ABI PRIZM 377 DNA sequencer. The sequences were analyzed by DNASTAR 5.0 package.

Statistical analysis

Based on the genotype number in analyzed breeds, genotypic frequencies and allelic frequencies of chemerin locus were calculated directly; Hardy-Weinberg equilibriums and differences in genotypic frequencies were analyzed using χ^2 test, which were performed by SPSS software (version 17.0). Population genetic indexes: He (gene heterozygosity), Ne (effective allele numbers) and PIC (polymorphism information content) were calculated according to Nei and Roychoudhury (1974) and Nei and Li (1979), respectively.

The software SPSS (version 17.0) was used to analyze the relationship between the genotypes and records of traits (UBF, ULMA and UMAR) on 214 Qinchuan individuals, which were measured by ultrasound, according to the following statistical linear model:

$$Y_{ijkl} = \mu + A_i + G_j + S_k + BF_l + \varepsilon_{ijkl} \quad (1)$$

Meat quality traits (BFT, EMA, MAR, WHC, MC and TD) were also evaluated by the comparison between the genotypes of 69 Qinchuan individuals and their phenotypic data by the least-squares method according to the following statistical linear model:

$$Y_{ijkl} = \mu + A_i + G_j + S_k + \varepsilon_{ijkl} \quad (2)$$

Where, Y_{ijkl} is the observation for the traits; μ is the overall population mean; A_i is the fixed effect of the ith age; G_j is the fixed effect of jth genotype (AA, AG and GG genotype); S_k is the fixed effect of sex; BF_l is the fixed effects of breed and farm and E_{ijk} is the random error.

RESULTS

Genetic polymorphism of Bos bovine chemerin gene

The 196 and 288 bp fragments of bovine chemerin gene

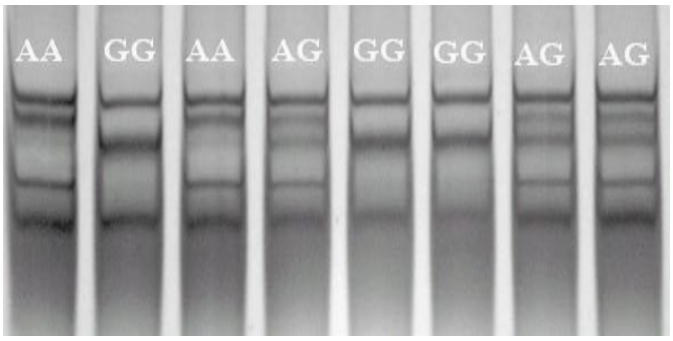

Figure 1. The electrophoresis patterns of PCR-SSCP exon 2 of bovine chemerin gene.

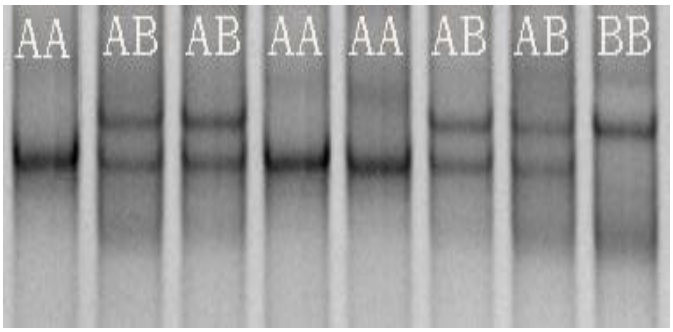

Figure 2. The electrophoresis patterns of PCR-SSCP exon 5 of bovine chemerin gene.

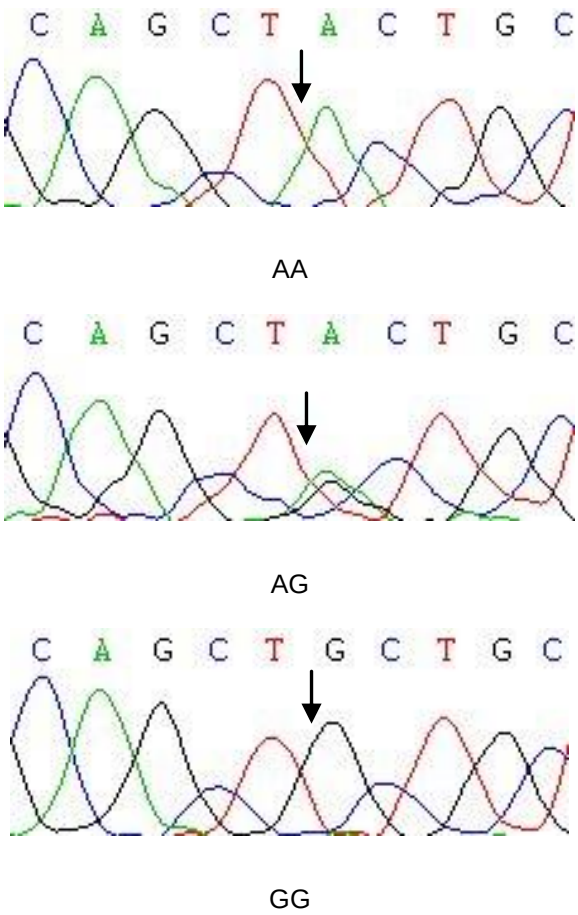

Figure 3. The sequencing maps of the novel SNP for locus 868A>G of the chemerin gene. Sample chromatograms of heterozygous (AG) and homozygous (AA and AG) genotypes are shown. The arrow denotes the location of the polymorphism.

of exon 2 and exon 5 were amplified by PCR. Then, by sequencing, two SNPs were revealed. They were synonymous mutation of leucine and aspartic acid, respectively. An adenine (A)-to-guanine (G) transition and an adenine (A) cytosine (C)-to-thymine (T) transition (868A>G and 2692C>T) were shown in two SNPs. The genetic polymorphisms of the six bovine breeds were detected by SSCP in the locus of 868A>G (Figure 1) and 2692C>T (Figure 2). The polymorphism of 868A>G locus was induced by A-G SNP at nucleotide 868 bp (Figure 3), and the polymorphism of 2692C>T locus was induced by C-T SNP at nucleotide 2962 bp of chemerin gene (Figure 4).

Genotypic, allelic frequencies and genetic characters in the six bovine breeds

The allele and genotype frequencies of the 868A>G and 2692C>T polymorphisms obtained for the different genetic groups are shown in Tables 1 and 2, respectively. The two alleles of the 868A>G polymorphism were observed in all genetic groups analyzed. Frequency of allele G was the predominant allele of locus 868A>G, expect for CYR and NY breeds. And three genotypes (named AA, AG and GG) were detected in all genetic groups; with respect to the 2692C>T polymorphism, both alleles were detected in the sample of animals studied. Allele A had significant lower frequent in the CYR group as compared to the other detected groups.

The χ^2-test showed that the genotype distributions in the detected breeds were in agreement with Hardy-Weinberg equilibrium (PHW value > 0.05) of locus 868A>G, except CYR breed, while XN breed was not at locus 2692C>T (PHW value < 0.05). The χ^2-test showed that the genotype distributions in the detected breeds were in agreement with Hardy-Weinberg equilibrium ($P > 0.05$), except CYR ($P < 0.01$) and XN ($P < 0.05$). This observation may be as a result of the occurrence of strict choice made by the people for forming the CYR and XN breed. According to the classification of PIC, all *Bos taurus* population belongs to the median polymorphism

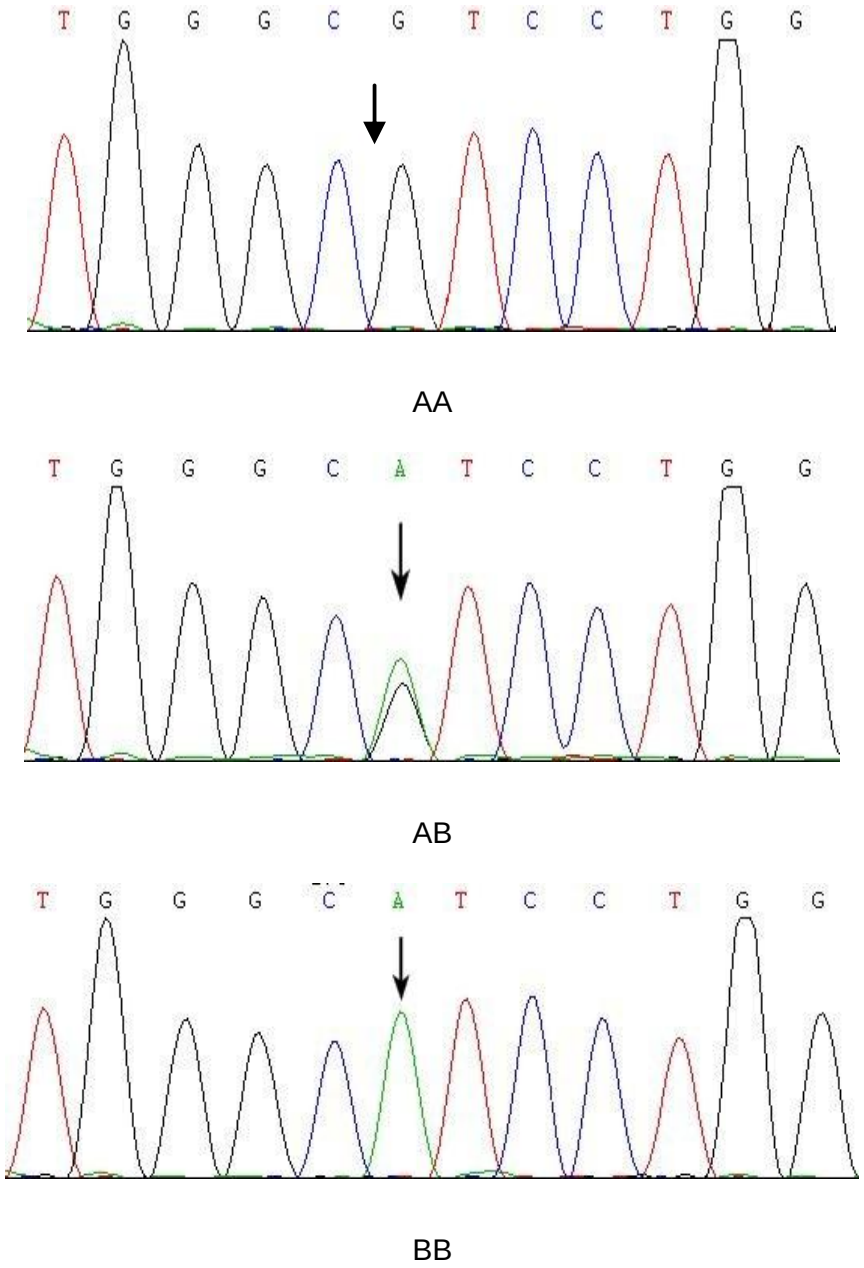

Figure 4. The sequencing maps of the novel SNP for locus 2692C>T of the chemerin gene. This figure was the reverse sequencing map of the locus 2692C>T; Sample chromatograms of heterozygous (AB) and homozygous (AA and BB) genotypes are shown. The arrow denotes the location of the polymorphism.

level and there was no significant difference of PIC value in the six breeds (Table 3).

Effect of the polymorphism locus on carcass traits

Association studies between each SNP genotypes and studied traits in Qinchuan cattle were given in Tables 4 and 5. In polymorphism locus 868A>G, animals with the genotype AA have higher mean values of UBF and ULMA than those with genotype AG ($P < 0.05$). While the association between genotypes and carcass and meat quality traits were analyzed, from which we can see significant differences on the LEA ($P < 0.05$) and WHC ($P < 0.05$) among different genotypes. Animals of AA genotype have greater mean values for BFT, LEA and WHC than those with AG genotypes. For locus 2692C>T SNP genotypes, the cattle with the AA genotype showed

Table 1. Genotype frequencies (%) of the locus 868A>G of chemerin gene in Bos bovine populations.

Breed	Observed genotype(number)			Total	Allelic frequency		χ^2(HW)	P_{HW} value
	AA	AG	GG		A	G		
QC	0.2150(46)	0.5047(108)	0.2804(60)	214	0.4673	0.5327	0.0401	0.9801
CYR	0.4911(55)	0.2679(30)	0.2411(27)	112	0.6250	0.3750	20.5714**	< 0.01
JR	0.1690(12)	0.4507(32)	0.3803(27)	71	0.3944	0.6056	0.2265	0.8927
LX	0.0870(6)	0.5942(41)	0.3188(22)	69	0.3841	0.6159	4.5198	0.1044
NY	0.3333(27)	0.4321(35)	0.2346 (19)	81	0.5494	0.4506	1.3124	0.5188
XN	0.1579(12)	0.5000(38)	0.3421(26)	76	0.4079	0.5921	0.0938	0.9542

HW, Hardy-Weinberg equilibrium; QC, Qinchuan cattle breed; CYR, Caoyuan red cattle breed; JR, Jiaxian red cattle breed; LX, Luxi cattle breed; NY, Nan yang cattle breed; XN, Xia'nan cattle breed. Generally, PHW value is classified into the following three types: in Hardy-Weinberg equilibrium (PHW value > 0.05), not in Hardy-Weinberg equilibrium (0.01 < PHW value < 0.05) and highly not in Hardy-Weinberg equilibrium (PHW value < 0.01).

Table 2. Genotype frequencies (%) of the locus 2692C>T of chemerin gene.

Breed	Observed genotype (number)			Total	Allelic frequency		χ^2(HW)	P_{HW} value
	AA	AG	GG		A	G		
QC	0.4626(99)	0.3972(85)	0.1402(30)	214	0.6612	0.3388	2.7539	0.2523
CYR	0.2679(30)	0.4018(45)	0.3304(37)	112	0.4688	0.5313	4.1839	0.1234
JR	0.4507(32)	0.4085(29)	0.1408(10)	71	0.6549	0.3451	0.6589	0.7193
LX	0.3913(27)	0.4348(30)	0.1739(12)	69	0.6087	0.3913	0.5259	0.7687
NY	0.3457(28)	0.3951(32)	0.2593 (21)	81	0.5432	0.4568	3.3686	0.1855
XN	0.3158(24)	0.3553(27)	0.3289(25)	76	0.4934	0.5066	6.3630*	0.0415

HW, Hardy-Weinberg equilibrium; QC, Qinchuan cattle breed; CYR, Caoyuan red cattle breed; JR, Jiaxian red cattle breed; LX, Luxi cattle breed; NY, Nan yang cattle breed; XN, Xia'nan cattle breed. Generally, PHW value is classified into the following three types: In Hardy-Weinberg equilibrium (PHW value > 0.05), not in Hardy-Weinberg equilibrium (0.01 < PHW value < 0.05) and highly not in Hardy-Weinberg equilibrium (PHW value < 0.01).

Table 3. Allele and genotype frequencies of the locus 868A>G and 2692C>T polymorphism of chemerin gene in the different genetic groups.

Locus	Breed	Gene heterozygosity	Effective allele number	PIC
868A>G	QC	0.4979	1.9915	0.3739
	CYR	0.4688	1.8824	0.3589
	JR	0.4777	1.9145	0.3636
	LX	0.4731	1.8979	0.3612
	NY	0.4951	1.9807	0.3725
	XN	0.4830	1.9344	0.3664
2692C>T	QC	0.4480	1.8117	0.3477
	CYR	0.4980	1.9922	0.3740
	JR	0.4520	1.8248	0.3498
	LX	0.4764	1.9097	0.3629
	NY	0.4963	1.9852	0.3731
	XN	0.4999	1.9997	0.3750

PIC, Polymorphism information content; QC, Qinchuan cattle breed; CYR, Caoyuan red cattle breed; JR, Jiaxian red cattle breed; LX, Luxi cattle breed; NY, Nan yang cattle breed; XN, Xia'nan cattle breed. Generally, PIC is classified into the following three types: Low polymorphism (PIC value < 0.25), median polymophism (0.25 < PIC value < 0.5) and high polymorphism (PIC value > 0.5).

greater UBF, BFT and WHC in comparison with the cattle with the AB and BB genotypes (P < 0.05).

Table 4. Association between locus 868A>G and 2692C>T genotypes of chemerin gene and UBF, ULMA and UMAR traits in Qinchuan cattle.

Polymorphism	Genotype	Trait (Mean ± SE)		
		UBF (mm)	ULMA (cm^2)	UMAR
868A>G	AA	0.980±0.3[a]	74.741±1.483[a]	2.718±0.055
	AG	0.900±0.22[b]	70.516±1.068[b]	2.585±0.040
	GG	0.925±0.2[ab]	73.460±1.170[ab]	2.646±0.043
	P value	0.104	0.042	0.145
2692C>T	AA	0.982±0.023a	73.044±1.240	2.653±0.042
	AB	0.898±0.025b	74.250±1.337	2.550±0.046
	BB	0.895±0.042ab	72.999±2.242	2.695±0.076
	P value	0.031	0.779	0.144

[ab] Means with different superscripts are significantly different ($P < 0.05$).

Moreover, the A>G synonymous mutation of leucine results in the increase of the part of the phenotypic variation, especially on the BFT and LEA phenotypes in animals studied, and the C>T synonymous mutation of aspartic acid results in the higher BFT phenotypes.

DISCUSSION

Although, initially chemerin was reported to play an important role in the innate and adaptive immunity (Parolini et al., 2007), recent researches gave a new point that chemerin play a crucial role in adipocyte metabolism, differentiation, obesity and diabetes in human and mice (Sell et al., 2009) and is related to the pathogenesis of metabolic syndrome (Michiko et al., 2008). Chemerin and its receptor ChemR23 are abundantly expressed in mouse and human adipose tissue (Thamer et al., 2008). Furthermore, chemerin is a well-known target gene of the retinoic acid receptor α (Müssig-K et al., 2009), and a growing body of evidence supports a link between retinoic acid signalling and adipocyte differentiation (Safonova et al., 1994). A recent study showed that the human chemerin gene have three SNPs, in which rs10278590 is associated with increased visceral fat mass in non-obese subjects of human, and in generalized obesity, this genetic effect may be masked by the close association between whole-body obesity and visceral fat mass (Müssig et al., 2009). The cloning and expression analysis of chemerin and chemerin receptor in Japanese Black cattle showed that bovine chemerin mRNA was highly expressed in the adipose and liver tissues, and is the TNF-α-up-regulated gene with a role in adipogenesis (Song et al., 2010). Above all, the available association studies on bovine and other livestock have never been reported. Therefore, we detected the SNPs of this gene in cattle, and found two SNPs (868A>G and 2692C>T) in exon 2 and exon 5, respectively.

Statistical analysis revealed that the chemerin gene polymorphisms of 868A>G had a significant effect on BFT and LEA in the Qinchuan cattle population studied, and the additive genetic effects were significant for WHC as well. Similarly, the polymorphisms of 2692C>T were significant on BFT and WHC. Therefore, we assumed that the mutation for 868A>G and 2692C>T might influence the carcass and meat quality traits; it could be a candidate molecular marker for the quality improvement of Qinchuan cattle. The meat quality traits of bovine are affected by many factors, such as genotype, breed, herd, location, sex and other random environmental factors. We got a new statistical model in which the three factors (breed, herd and location) were involved and then, we employed the least-squares method in GLM procedure of SPSS software to do the related analysis and we did not find any significant difference ($P > 0.05$) (data not shown).

Ultrasound technology has been popularly used in recent years, for example, Trejo et al. (2010) used the ultrasound technology to measure the backfat and marbling deposition in feedlot cattle to evaluate the different effect on the ultrasound backfat and marbling deposition. Previously, Hamlin et al. (1995) indicated that ultrasonic predictors showed about 10% less variation in retail product percentage than did carcass measurements. Greiner et al. (2003) found that the ultrasound measurements were useful predictors of retail yield in live animal, such as 12th-rib fat thickness and longissimus muscle area. Our result showed that the relevance in this study was the same as the traits measured by ultrasound with the carcass traits (Jiao et al., 2010).

Implications

Our data showed that the chemerin gene might have potential influence on carcass and meat quality traits in Qinchuan cattle. And the impact of SNPs on these traits variability represents a vast area for further research. It is also significant to investigate whether the chemerin gene

Table 5. Association between locus 868A>G and 2692C>T genotypes of chemerin gene and carcass and meat quality traits in Qinchuan cattle.

Polymorphism	Genotype	Trait (Mean ± SE)						
		Live weight (LW)/kg	Carcass weight (CW)/kg	Backfat thickness (BFT) (cm)	Loin eye area (LEA)/cm^2	Marbling score (MS)/1-5	Meat tenderness (MT)/kg	Water holding capability (WHC)/%
868A>G	AA	400.765±9.511	198.035±8.956	1.214±0.086^a	81.251±4.344^a	2.647±0.127	2.154±0.136	79.788±1.539^a
	AG	388.333±7.160	185.587±6.741	1.013±0.065^b	69.004±3.270^b	2.333±0.095	2.062±0.102	74.523±1.159^b
	GG	397.500±8.005	189.733±7.537	1.087±0.072^{ab}	78.555±3.656^{ab}	2.500±0.107	2.230±0.115	75.083±1.295^b
	P	0.521	0.542	0.064	0.048	0.138	0.548	0.021
2692C>T	AA	392.103±6.892	193.738±6.239	1.215±0.058a	73.896±3.452	2.552±0.100	2.013±0.105	78.348±1.260a
	AB	396.208±7.576	185.967±6.859	1.027±0.064b	77.404±3.795	2.375±0.110	2.293±0.115	74.288±1.385b
	BB	389.444±12.371	183.044±11.20	1.014±0.104ab	74.175±6.197	2.556±0.179	2.289±0.188	73.433±2.261ab
	P	0.872	0.593	0.061	0.777	0.452	0.160	0.050

^{ab} Means with different superscripts are significantly different ($P < 0.05$).

plays a role in the development of these traits and whether it involves linkage disequilibrium with other causative mutations. In conclusion, ultrasound technology could be a useful method in animal production, especially in breeding, and people can manage their farm easily.

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