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# Polymorphisms of the bovine chemokine receptor-like 1 gene and their associations with meat quality traits in Qinchuan cattle (*Bos taurus*)

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## Abstract

**Background:** We investigated the polymorphisms of the bovine chemokine receptor-like 1(CMKLR1) gene. The coding region of CMKLR1 was screened in Qinchuan cattle by PCR-RFLP technology.
**Results:** In this study, we discovered two single nucleotide polymorphisms (SNPs) (264G > C and 762C > T) in the coding region of the CMKLR1 gene. Hence, we described the BmgT120I and Pdm1 PCR-RFLP methods for detecting the 64G > C and 762C > T mutations, respectively. PCR-RFLP and sequencing were used to analyze the two loci of CMKLR1 gene in 324 individuals, which were randomly selected from breeding populations. Furthermore, meat quality traits in another 80 Qinchuan individuals were analyzed by the comparison between the genotypes and their phenotypic data.
**Conclusions:** The results showed that the G264C SNP and C762T SNP of bovine CMKLR1 were significantly associated with backfat thickness (BFT) and water holding capacity (WHC), respectively.

**Keywords:** CMKLR1 gene, meat quality traits, PCR-RFLP, Qinchuan cattle, SNP.

## INTRODUCTION

Chemokine-like receptor (CMKLR1), also known as ChemR23 (chemerin receptor 23), is a G-protein-coupled receptor with seven-transmembrane α-helical structure (Yoshimura and Oppenheim, 2011). Recent studies identified ligands that specifically bind to this receptor and began to reveal their contribution to the regulation of immune responses and other cellular processes (Wittamer et al. 2003; Vermi et al. 2005; Zabel et al. 2005a; Zabel et al. 2005b). Chemerin expression and secretion has been shown to increase dramatically with adipocyte differentiation (Bozaoglu et al. 2007; Goralski et al. 2007). Moreover, it was reported that loss of chemerian expression almost abrogates adipogenesis (Goralski et al. 2007). Chemrin as a novel adipokine with potential autocrine was strongly expressed in white adipose tissue (Bozaoglu et al. 2007; Goralski et al. 2007). These results suggest adipocyte differentiation has a connection with CMKLR1 gene expression.

To our knowledge, there is no related information on polymorphism of bovine CMKLR1 gene be studied so far. Based on the important roles of CMKLR1 in adipocyte differentiation, CMKLR1 could be an attractive candidate gene for meat quality traits in bovine. Therefore, the objective of this study is to detect SNPs of bovine CMKLR1 gene and to explore their possible association with meat quality traits in *Bos taurus*.

## MATERIALS AND METHODS

### DNA samples and data collections

Blood samples from 324 Qinchuan cattle were randomly selected from breeding populations and used to analyze the CMKLR1 allelic frequencies. Apart from that, 80 QC steers of 1.5 to 2 years old were selected randomly and slaughtered to collect meat quality traits, including Backfat thickness (BFT), Loin-eye area (LEA), Marble score (MAR), Water holding capacity (WHC) and Tenderness (TD). In order to minimize systematic error, single person was assigned to measure one of the five meat quality traits in all animals.

Genomic DNA samples were obtained from the 324 animals. DNA samples were extracted from leukocytes and tissue samples using, standard phenol-chloroform protocol (Mullenbach et al. 1989).

### PCR amplification and sequencing

Two pairs of polymerase chain reaction (PCR) primers (Table 1) were designed to amplify the coding and flanking regions of bovine CMKLR1 (GenBank accession number NC_007315.3). Polymerase chain reaction (PCR) amplifications were performed in 20 μL reaction mixture containing 50 ng DNA, 10 pM of each primer, 0.20 mM dNTP, 2.5 mM $MgCl_2$ and 0.5 U taq DNA polymerase (TaKaRa, Dalian, China). Seven PCR reactions were performed using different DNA templates from the seven breeds respectively. The cycling protocol was 5 min at 95ºC, 32 cycles of denaturing at 94ºC for 30 sec, annealing at 64ºC for 30 sec, extension at 72ºC for 30 sec, followed by a final extension for 10 min. The products for sequencing were purified with Axygen kits (BMI fermentas) and sequenced in both directions in an ABI PRIZM 324 DNA sequencer (Perkin-Elmer). The sequences were analyzed with SeqMan software.

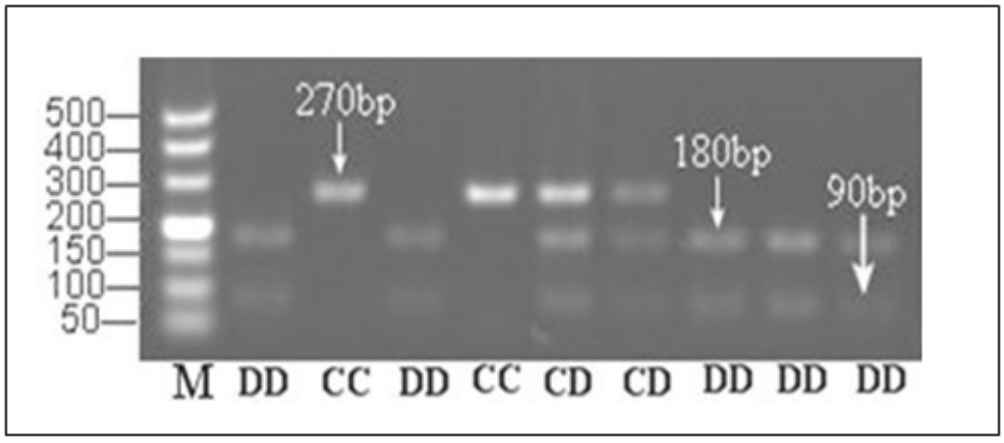


**Fig. 1 Agarose gel electrophoresis of Taq IPCR-RFLP.** Note: CC genotype shows one fragment (270 bp), CD genotype shows three fragments (270, 180 and 900 bp) and DD genotype shows two fragments (180 and 90 bp).

### Genotyping of CMKLR1 allele by PCR-RFLP

Aliquots of 20 μL *C* Exon C PCR products were digested with 10 U BmgT120l (TaKaRa, Dalian, China) at 37ºC for 6 hrs following the supplier manual and 20 μL *C* Exon D PCR products were digested with 10 U Pdm1 (TaKaRa, Dalian, China) at 37ºC for 4 hrs. The digested products were detected by electrophoresis in 1.5% agarose gel stained with ethidium bromide. To confirm the results based on the PCR-RFLP technique, the PCR products of different electrophoresis patterns were sequenced in both directions.

**Statistical analysis**

Based on the genotype number of the CMKLR1 and its flanking region locus in analyzed breeds, the following items were statistically analyzed according to the previous approaches (Nei and Roychaudhury, 1974; Nei and Li, 1979; Liu et al. 2010), including genotypic frequencies, allelic frequencies.

$Y_{ijk} = \mu + G_i + S_j + Age_k + E_{ijk}$

Hardy-Weinberg equilibrium, gene homozygosity, effective allele numbers and polymorphism information content (PIC). Meat quality traits (BFT, EMA, MAR, WHC, MC, and TD) were also evaluated by the comparison between the genotypes of 80 Qinchuan individuals and their phenotypic data by the least-squares method according to the following statistical linear model where $Y_{ijk}$ is observation for the meat quality traits, μ is overall mean for each trait, $G_i$ is genotype effect, $S_j$ is fixed effect of sex, $Age_k$ is regression variable for measure age, $E_{ijk}$ is random environment effect.

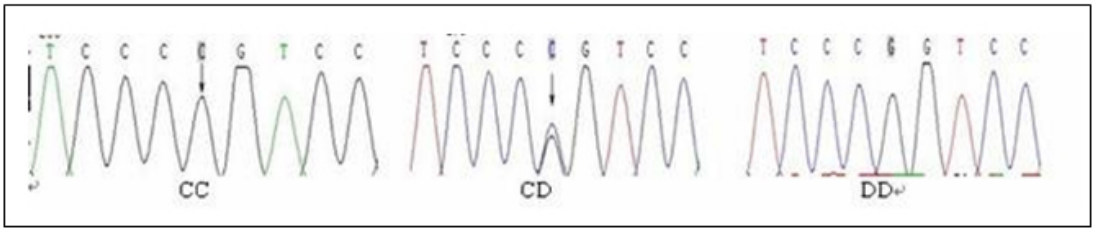


**Fig. 2 Agarose gel electrophoresis of Taq IPCR-RFLP.** Note: RR genotype shows one fragment (305 bp), RP genotype shows three fragments (305, 208 and 97 bp) and PP genotype shows two fragments (208 and 97 bp).

**RESULTS**

Based on RFLP analysis and DNA sequencing methods, the bovine CMKLR1 gene showed two SNPs. After a 270 bp product of *C* Exon C was amplified and sequenced, we identified the first SNP, named G264C. It is a synonymous mutation of proline which creates the BmgT120I restriction site (G^GNC). In the *C* Exon D, a 305 bp product was sequenced, we also found a SNP named C762T. It is a synonymous mutation of tyrosine which creates the Pdm1 restriction site (GAANN^NNTTC).

For the G264C SNP in the Qinchuan cattle that we analyzed, three size variants of restriction fragments were identified, namely: 270, 180 and 90 bp. Subsequently, we analyzed the localization of migration bands of the restriction fragments and found out three genotypes of “mutation G > C”. Figure 1 shows that the genotype CC represents the occurance of one band of 270 bp, genotype CD represents three restriction fragment bands of 270, 180 and 90 bp and genotype DD represents two restriction fragment bands of 180 and 90 bp. In the other C762T SNP, we also identified three size variants of restriction fragments, namely: 305, 208 and 97 bp. Figure 2 shows that the genotype RR represents the occurance of one band of 305 bp, genotype RP represents three restriction fragment bands of 305, 208 and 97 bp and genotype PP represents two restriction fragment bands of 208 and 97 bp. In order to better understand the detailed genetic variation within the Qinchuan cattle CMKLR1 gene, the polymorphic DNA amplification fragments were sequenced and the sequence data corresponded to the polymorphic patterns. The sequencing maps are shown in Figure 3 and Figure 4.

With the PopGen software (version 3.2) and according to Botstein’s method (Botstein et al. 1980), genotypic and allelic frequencies in the PCR-RLFP analysis with BmgT120I and Pdm1 were investigated and performed by $x^2$ test in our study (Table 2 and Table 3). The date showed here demonstrate that the frequencies of CMKLR1-D allele (0.6034) and CMKLR1-R allele (0.5478) were in the ascendant in the allelic frequency of Qinchuan cattle. The frequencies of CD genotype was 0.5401 in *C* Exon C, and the frequencies of RP genotype was 0.4290 in *C* Exon D, these suggested that the CD genotype and RP genotype were dominant. Genehozygosity of the two SNPs (0.5214 for G264C, 0.5046 for C762T) were higher than geneheterozygosity in Qinchuan cattle.

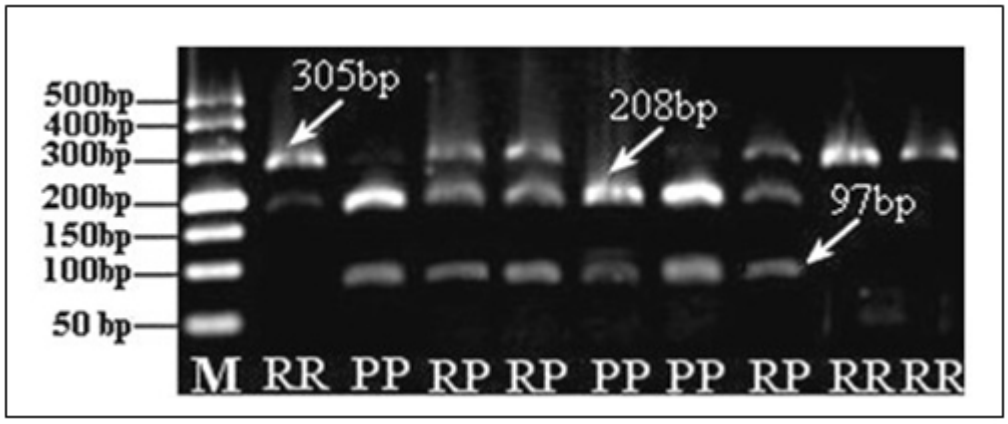


**Fig. 3 Sequencing chromatograms of CC, CD and DD genotypes of chemerin gene exon 5.**

Generally, PIC (Polymorphism Information Content) is classified in to the following three types: low polymorphism (PIC value < 0.25), median polymorphism (0.25 < PIC value < 0.5) and high polymorphism (PIC value > 0.5). According to this classification of PIC, Qinchuan cattle belongs to the median polymorphism level and this suggests that Qinchuan cattle was in Hardy-Weinberg equilibrium (Table 2 and Table 3).

Then, we analyzed the association of BmgT120I and Pdm1PCR-RFLPs with meat quality traits, including BFT, LEA, MAR, WHC and TD, in another 80 QC individuals. The results are shown in Table 4 and Table 5 from which we can see there are significant differences on the BFT ($P < 0.05$) and MAR ($P < 0.01$) in G264C locus among different genotypes. These also imply that there are significant differences on the WHC ($P < 0.05$) and MAR ($P < 0.05$) in C762T locus among different genotypes. In G264C locus, animals of CC genotype have higher mean values for BFT than these with CD and DD genotypes, but they have the lowest level of MAR. In C762T locus, animals of RR genotype have higher mean values for WHC than these with RP and PP genotypes. Moreover, animals of RP genotype have higher mean values for MAR than these with RR genotypes. No other significant correlation was observed between any of the marker genotype at G264C or C762T and other traits.

Moreover, in G264C locus, the G > C synonymous mutation of proline results in the increase of the part of the phenotypic variation, especially on the BFT and MAR phenotypes in animals studied. In C762T locus, the C > T synonymous mutation of tyrosine results in the increase of the part of the phenotypic variation, especially on the WHC and MAR phenotypes in animals studied. Therefore, we assume that the mutation for G264C and C762T could have an important influence on many minor genes which involved in BFT and WHC, respectively.

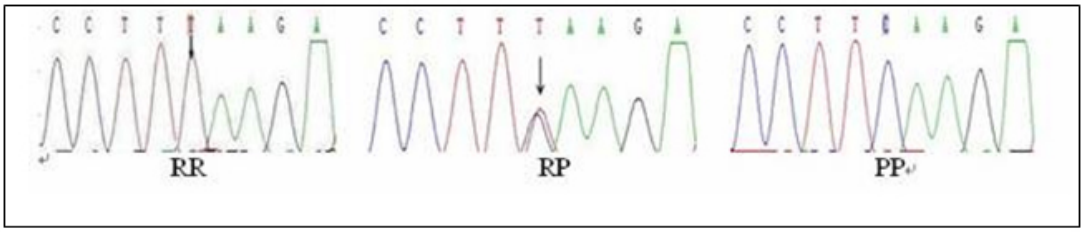


**Fig. 4 Sequencing chromatograms of RR, RP and PP genotype of C762T location**.

## DISCUSSION

As we have pointed out that, CMKLR1 has multiple roles in adiopcyte differentiation and function. Several studies have shown that chemerin gene SNP is associated with increased visceral fat mass

(Müssig et al. 2009). A few reports pointed out chemerin gene is associated with carcass traits in cattle (Tian et al. 2011). It has been known that the close association between whole-body obesity and visceral fat mass can mask this genetic effect in generalized obesity (Müssig et al. 2009). Above all, although many studies focus on CMKLR1 gene functions, the available association studies on bovine and other livestock have never been reported.

We supposed that CMKLR1 gene is connected with the content of fat in muscle and correlated with the carcass traits such as EMA, TD, BFT, WHC and MC in Qinchuan cattle. However, no further research was carried out on the relationship of the CMKLR1 gene with carcass traits in animals, so we studied the SNPs of this gene in cattle and determined their relevance. The present study firstly shows that the G264C SNP and C762T SNP of bovine CMKLR1 are significantly associated with BFT and WHC, respectively, in bovine (Table 3 and Table 4).

Recently, it was reported that synonymous SNPs can affect protein expression (Nackley et al. 2006). Therefore, we hypothesized that synonymous G264C and C762T might have similar biological functions, including alteration of mRNA stability, modulation of the efficiency of translation of mRNA, and consequently influence of encoded protein structure and property, which might affect directly or indirectly the production traits of domestic animals (Capon et al. 2004).

In conclusion, we identified two SNPs in the CMKLR1 gene. Our results provided evidence that the CMKLR1 gene might have potential effects on meat quality traits in bovine. Therefore, further work will be necessary to use the SNP for marker-assisted selection (MAS) in larger populations. It is also significant to investigate whether the CMKLR1 gene plays a role on development of those traits and whether it involves in linkage disequilibrium with other causative mutations.

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# Tables

**Table 1. Primer sequences ofCMKLR1 gene for PCR amplifications in Qinchuan cattle.**

| Primer | Primer sequences (5'to 3') | | Product size |
|---|---|---|---|
| *C* Exon C | ATGGAAGGCAGAGTGGTC | CGTGTTGTGGATGAGCAG | 270 |
| *C* Exon D | GCGCTTCCTCTGTGGCT | TCTTGAAGTCCTGACCC | 305 |

**Table 2. Association between G264C SNP genotypes of CMKLR1 gene and polymorphism of CMKLR1 gene PCR-AsuI-RFLP in Qinchuan cattle.**

| Sample | Genotype frequency | | | Allele frequency | | Homozygosity | Ne | PIC | $X^2$ |
|---|---|---|---|---|---|---|---|---|---|
| | CC | CD | DD | C | D | | | | |
| 324 | 0.1265 (41) | 0.5401 (175) | 0.3333 (108) | 0.3966 | 0.6034 | 0.5214 | 1.9180 | 0.3641 | 5.3503 |

Note: $X^2_{0.05} = 5.99$, $X^2_{0.01} = 9.21$, d.f. = 2. If $X^2 < X^2_{0.05}$, means the polymorphism was in Hardy-Weinberg equilibrium. If $X^2_{0.05} < X^2 < X^2_{0.01}$, means the polymorphism was not in Hardy-Weinberg Balance. If $X^2 > X^2_{0.01,}$ means the polymorphism was extremely not in Hardy-Weinberg equilibrium not in Hardy-Weinberg equilibrium.

**Table 3. Association between C762T SNP genotypes of CMKLR1 gene and polymorphism of CMKLR1 gene PCR- Pdm1-RFLP in Qinchuan cattle.**

| Sample | Genotype frequency | | | Allele frequency | | Homozygosity | Ne | PIC | $X^2$ |
|---|---|---|---|---|---|---|---|---|---|
| | RR | RP | PP | R | P | | | | |
| 324 | 0.3333 (108) | 0.4290 (139) | 0.2377 (77) | 0.5478 | 0.4522 | 0.5046 | 1.9819 | 0.3727 | 5.8219 |

Note: Different letters in the same row means significant difference between the treatments (P < 0.05); same letter in the same row means no significant difference between treatments (P > 0.05).

**Table 4. Association of polymorphism of G264C of CMKLR1gene with meat quality traits in Qinchuan cattle.**

| Trait | Genotypes | | | |
|---|---|---|---|---|
| | CC | CD | DD | P |
| MAR | $2.000^b \pm 0.159$ | $2.538^a \pm 0.080$ | $2.545^a \pm 0.107$ | <0.05 |
| LEA | 73.057 ± 6.397 | 78.271 ± 3.239 | 78.591 ± 4.313 | >0.05 |
| BFT | $1.279^a \pm 0.098$ | $1.049^b \pm 0.049$ | $0.970^b \pm 0.066$ | <0.05 |
| MT | 2.253 ± 0.125 | 1.932 ± 0.063 | 1.933 ± 0.085 | >0.05 |
| WHC | 0.216 ± 0.026 | 0.202 ± 0.013 | 0.208 ± 0.017 | >0.05 |

Note: Different letters in the same row means significant difference between the treatments (P < 0.05); same letter in the same row means no significant difference between treatments (P > 0.05).

**Table 5. Association of polymorphism of C762T of CMKLR1gene with meat quality traits in Qinchuan cattle.**

| Trait | Genotypes | | | |
|---|---|---|---|---|
| | RR | RP | PP | P |
| MAR | $2.296^{b} \pm 0.103$ | $2.625^{a} \pm 0.095$ | $2.381^{ab} \pm 0.117$ | <0.05 |
| LEA | 78.969 ± 3.968 | 80.017 ± 3.645 | 79.172 ± 4.500 | >0.05 |
| BFT | 1.047 ± 0.071 | 1.096 ± 0.065 | 1.108 ± 0.080 | >0.05 |
| MT | 2.216 ± 0.111 | 2.139 ± 0.102 | 2.087 ± 0.126 | >0.05 |
| WHC | $0.266^{a} \pm 0.016$ | $0.245^{b} \pm 0.015$ | $0.248^{b} \pm 0.018$ | <0.05 |

Note: Different letters in the same row means significant difference between the treatments ($P < 0.05$); same letter in the same row means no significant difference between treatments ($P > 0.05$).