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Assessment of haplotype variation in bovine *AMPD1* gene for association with growth and carcass traits in Qinchuan beef cattle

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Abstract

The *AMPD1* gene plays an important role in the purine nucleotide cycle and energy metabolism in skeletal muscle. In the present study polymorphisms of the *AMPD1* gene were detected by PCR-SSCP and DNA sequencing of 215 individuals of the Qinchuan beef cattle breed. DNA sequencing revealed two mutations by comparisons with the bovine genome sequence (acc. no.: NC_007301). Two single nucleotide polymorphisms (SNPs; g.19416T>C and g.19421A>G) were detected in intron 11 of the bovine *AMPD1* gene. The sequencing of PCR products of animals providing different PCR-SSCP banding patterns showed that four kinds of haplotypes, named: A (T-A), B (T-G), C (C-A) and D (C-G); and the five diplotypes were segregating: AA (T-A/T-A), BC (T-G/C-A or C-G/T-A), AC (T-A/C-A), CC (C-A/C-A) and CD (C-A/C-G). A significant association of *AMPD1* with carcass weight was shown. Animals with the new heterozygote diplotype BC ($P<0.05$, n=56) had greater carcass weight than those with the other diplotypes. The SNPs in *AMPD1* may be used as a possible candidates for marker-assisted selection in Qinchuan beef cattle breeding program.

Keywords: *AMPD1* gene, mutation, haplotype, growth and carcass traits, Qinchuan beef cattle

Zusammenfassung

Bewertung der Haplotyp Variation bei Rindern *AMPD1* Gen für Vereinigung mit Wachstums- und Schlachtleistung in Qinchuan Rinder

Das *AMPD1*-Gen spielt eine wichtige Rolle im Purinnukleotid- und Energiestoffwechsel des Skelettmuskels. In der vorliegenden Studie wurden durch PCR-SSCP und DNA-Sequenzierung von 215 Tieren der Fleischrindrasse Qinchuan nach Polymorphismen das *AMPD1*-Gen detektiert. Das Ergebnis der DNA-Sequenzierung zeigte zwei Mutationen durch Vergleiche mit der genomischen Sequenz des Rindes (acc. no.: NC_007301). Die Polymorphismen (single nucleotide polymorpjhisms, SNPs; g.19416T>C und g.19421A>) wurden im Intron 11 des bovinen *AMPD1*-Gens nachgewiesen. Die Sequenzierungsergebnisse von PCR-Produkten von Tieren mit unterschiedlichen PCR-SSCP- Bandenmustern ergaben, dass vier Haplotypen – A (TA), B (TG), C (CA) und D (CG) – und fünf Diplotypen segregierten: AA (TA/TA), BC (TG/CA oder CG/TA), AC (TA/CA), CC (CA/CA) und CD (CA/CG). Eine signifikante

Assoziation von *AMPD1* mit dem Schlachtkörpergewicht konnte gezeigt werden. Tiere mit dem neuen heterozygoten Diplotyp BC ($P<0,05$, n=56) erreichten ein höheres Schlachtgewicht als Tiere mit den anderen Diplotypen. Die SNPs im *AMPD1* erweisen sich damit als mögliche Kandidaten für die markergestützte Selektion im Qinchuan-Rinder-Zuchtprogamm.

Schlüsselwörter: *AMPD1* Gen, Mutation, Haplotyp, Wachstum und Schlachtleistung, Qinchuan Rinder

Introduction

Adenosine monophosphate deaminase 1 (*AMPD1*) is a highly active enzyme in the skeletal muscle that plays an important role in the adenine nucleotide catabolism (Morisaki *et al*. 1990). Adenosine monophosphate deaminase 1 catalyzes the conversion of adenosine monophosphate to inosine monophosphate. Subsequent cloning of three human genes has revealed the molecular basis for four different isoforms: AMPD1, isoforms M, muscle; AMPD2, isoforms L, liver; AMPD3, isoforms E1 and E2, erythrocyte (Sabina *et al*. 1990, Bausch *et al*. 1992, Mahnke *et al*. 1992, Mahnke *et al*. 1996). It is likely that the three AMPD genes arose from duplication of a common primordial gene (Morisaki *et al*. 1990), and subsequently, acquired differences via divergent evolution. Consistent with this hypothesis, AMPD isoforms contain both conserved and divergent domains. The three AMPD polypeptides share a similar 550 amino acid C-terminal end (62-70 % identical) that contains a motif signature sequence believed to be the catalytic center of the enzyme (Chang *et al*. 1991, Gross *et al*. 1994). Conversely, each AMPD polypeptide differs by divergent N-terminal sequences of 200-330 amino acids with less than 36 % identity to each other. In addition, differential promoter use and alternative splicing add extensions or substitutions of four (AMPD1), 47-128 (AMPD2) (Mineo *et al*. 1990, Van *et al*. 1995), and 7-9 (AMPD3) amino acids at the distal N-terminal end of each AMPD polypeptide. Available information suggests that different N-terminal domains and distal N-terminal variations in each AMPD polypeptide contribute to isoform-specific behaviors of this enzyme (Sabina *et al*. 2000).

The skeletal muscle-specific isoform (M) of AMPD is encoded by the *AMPD1* gene, located on the short arm of chromosome 1 (Sabina *et al*. 1990). This isoform accounts for more than 95 % of the total AMPD in muscle (Fishbein *et al*. 1993). It is mainly located in type II muscle fibers particularly at the neuromuscular junction, but also in capillaries (Van *et al*. 1994).

The porcine *AMPD1* gene was mapped to SSC 4q1.6-q2.3 (Stratil *et al*. 2000). In early studies reported that the porcine *AMPD1* maps within known QTL (quantitative trait locus) with effects on carcass traits such as carcass weight, loin and neck meat weight, loin muscle area, shoulder meat weight, ham meat weight, chops weight. Therefore, porcine *AMPD1* gene may be an important candidate gene of body measurement and carcass traits and the association results in our study indicated that the SNPs may simply be used as genetic markers linking to quantitative trait loci with effects on carcass traits. Further investigation is required among other populations of pigs to confirm the association between the PCR-Eco81I-RFLP and carcass traits (Wang *et al*. 2008). The *AMPD1* gene might be a candidate gene of meat production trait and provides useful information for further studies on its roles in porcine

skeletal muscle, etc. Up to now, bovine *AMPD1* gene is blank research regarding growth and carcass traits. Therefore, we focus on bovine *AMPD1* gene, which could be candidate genes of bovine growth and carcass traits.

At present, no study has revealed any genetic information relevant to bovine *AMPD1* gene. The goal of our study was to identify sequence variation of *AMPD1* gene in Qinchuan cattle breed, and to analyze the relationship between gene variation and growth and carcass traits.

Materials and methods

Animal source and DNA preparation

In this study, a total of 215 beef cattles belonging to Qinchuan (QC) cattle populations, were randomly selected from commercial populations and used in the association analysis. The animals (30±2 months of age at slaughter) were reared in the province of Shaanxi, China. The records of growth traits (body length, body height, and hip width) and carcass traits (slaughter weight, carcass weight, dressing percentage) were measured according to the criterion GB/T17238-1998 Cutting Standard of Fresh and Chilled Beef in China (China Standard Publishing House). All experimental procedures were performed according to authorization granted by the Chinese Ministry of Agriculture.

Genomic DNA samples were obtained from 215 beef cattles were isolated from 2 % heparin-treated blood samples and stored at −80 °C, following the standard procedures (Sambrook *et al*. 2002).

Primer design and PCR amplification conditions

Primers used to amplify bovine *AMPD1* gene intron 11 locus were designed from a published gene sequence (GenBank acc. no: NC_007301). The sequences of the primers as follows:

F: 5′-AAC CCT CAG GCT CAC CCA-3′ (nt 19273-19290);
R: 5′-GGG CTT AGG GCT CTT GGA -3′ (nt 19550-19567).

The size of expected PCR products was 299 bp, containing the whole intron 11 and parts of the exon 11 and exon 12 regions.

The 25 µL volume contained: 50 ng genomic DNA, 0.5 µM of each primer, 1×Buffer (including 1.5 mM $MgCl_2$), 200 µM dNTPs and 0.625 units of Taq DNA polymerase (MBI). The cycling protocol was 5 min at 95 °C, 35 cycles of 94 °C for 30 s, 60 °C annealing for 30 s, 72 °C for 30 s, with a final extension at 72 °C for 10 min.

Single-stranded conformation polymorphism (SSCP)

Aliquots of 5 µL PCR products were mixed with 5 µL denaturing solution (95 % formamide, 25 mM EDTA, 0.025 % xylene-cyanole, and 0.025 % bromophenol blue), heated at 98 °C for 10 min, and immediately chilled on ice. Denatured DNA was subjected to 10 % polyacrylamide gel electrophoresis (PAGE) (80×73×0.75 mm) in 1×TBE buffer and constant voltage (200 V) for 2 h at a constant temperature of 4 °C. The gel was stained with 0.1 % silver nitrate (Zhang *et al*. 2007).

DNA sequencing analysis

The PCR products from different PCR-SSCP genotypes were purified by using the DNA Fragment Purification Kit (BIODEV Corp., Beijing, P. R. China) and sequenced in both directions (Beijing Aolaibo Biotechnology, P. R. China; Applied Biosystems 3730xl DNA sequencer, Foster city, CA, USA); Sequences were analyzed with BioXM software (Version 2.6).

Statistical analysis

Gene frequencies were determined for Qinchuan cattle breed by direct counting. Chi-square tests (also chi-squared or χ^2 test) were used to determine if the individual variant was in Hardy-Weinberg equilibrium. Levels of genetic variability were estimated with the unbiased expected gene homozygosity (H_o), gene heterozygosity (H_e), the effective allele numbers (Ne), and the polymorphic information content (PIC). The formulas were as follows:

$$H_o = \sum_{i=1}^{n} P_i^2 \qquad H_e = 1 - \sum_{i=1}^{n} P_i^2 \qquad N_e = 1 / \sum_{i=1}^{n} P_i^2 \qquad PIC = 1 - \sum_{i=1}^{m} P_i^2 - \sum_{i=1}^{m-1} \sum_{j=i+1}^{m} 2P_i^2 P_j^2 \qquad (1)$$

where *Pi* is the frequency of the *i* allele, *n* is the number of alleles.

The traits were compared between the genotypes of bovine *AMPD1* gene. The relationship between genotypes and growth and carcass traits were analysed by the least-squares method as applied in the general linear model (GLM) procedure of the SPSS software (Version 16.0) according to the following linear model (Gan *et al*. 2008):

$$Y_{ijk} = \mu + M_i + G_j + e_{ijk} \qquad (2)$$

where Y_{ijk} is the observed value, μ is the overall mean for each trait, M_i is the fixed effect of *i*-th month of slaughtering, G_j is the fixed effect of *j*-th single SNP marker genoetype and e_{ijk} is the random error.

Results and discussion

Genotype patterns of different polymorphisms

The polymorphisms of bovine *AMPD1* gene were detected by PCR-SSCP and DNA sequencing methods. The results showed that two mutations in intron 11 in Qinchuan cattle breed. The SSCP results showed polymorphic information with five unique SSCP banding patterns observed in Qinchuan cattle population (Figure 1).

In order to better understand the detailed genetic variation within the Chinese bovine *AMPD1* gene. The polymorphic DNA amplification fragments were sequenced in both directions. The DNA sequence of the mutation has been submitted to the GenBank database (GQ861240), and mutation sequencing maps of five observed diplotypes are shown in Figure 2. The comparison between nucleotide sequence of bovine *AMPD1* gene (GenBank acc.no: NC_007301) and the GQ861240 sequence revealed two mutations: the NC_007301: g. 19416T>C and g. 19421A>G mutations.

Four haplotypes were described as: A (T-A), B (T-G) C (C-A) and D (C-G), respectively (Figure 2). Accordingly, nine diplotypes might be described as: AA (T-A/T-A), BB (T-G/T-G), CC (C-A/C-A),

DD (C-G/C-G), AB (T-A/T-G), AC (T-A/C-A), AD or BC (T-A/C-G or T-G/C-A), BD (T-G/C-G) and CD (C-A/C-G). With the sequence data from different individuals, the five diplotypes were conflated and described as: AA, BC, AC, CC and CD. These five diplotypes corresponded to five polymorphic patterns found in this study.

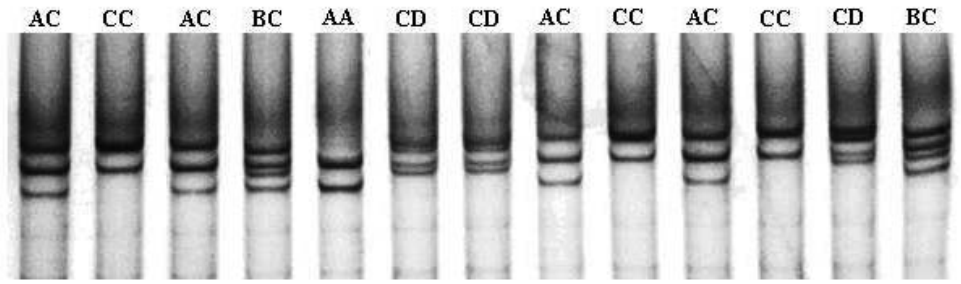

Figure 1
The PCR-SSCP patterns of the bovine *AMPD1* gene in 10 % PAGE. Note: Five unique SSCP patterns (AA, BC, AC, CC and CD) were observed in Qinchuan cattle population.

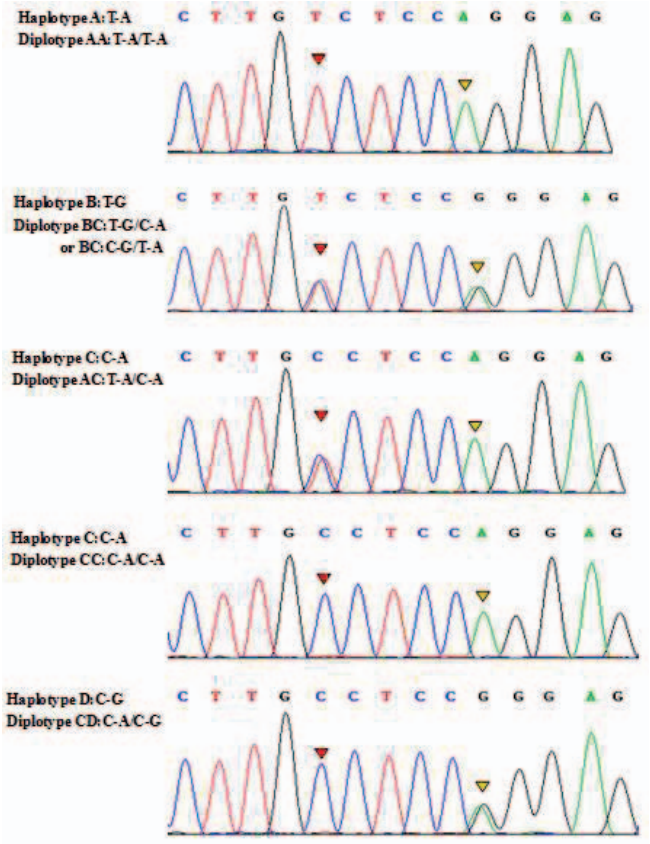

Figure 2
The sequencing determinations contain two novel mutations (T19416C and A19421G) in bovine *AMPD1* gene from different haplotypes and diplotypes.

Analysis of polymorphism of the AMPD1 gene in Qinchuan cattle breed

The frequencies of genotypes TT/TC/CC and AA/AG/GG in Qinchuan population were 0.1860/0.4791/0.3349 and 0.6186/0.3814/0.0000. The frequencies of allele T/C and A/G of Qinchuan populations were 0.4258/0.5744 and 0.8093/0.1097. In present population, the population genetic parameters of Ho, He, Ne and PIC were presented in Table 1. According to the classification of PIC (PIC value<0.25, low polymorphism; 0.25<PIC value<0.5, intermediate polymorphism; and PIC value>0.5, high polymorphism), Qinchuan cattle breed possessed high genetic diversity in two SNPs loci, this reflected that there was a very high genetic diversity within Chinese bovine *AMPD1* gene in the analyzed population.

The χ2-test showed that the genotype distributions of Qinchuan cattle in Hardy-Weinberg equilibrium (P>0.05) at A19416G locus, and disagreement with at Hardy-Weinberg equilibrium (P<0.05) at A19421G locus, which showed that there was not a dynamic equilibrium even in artificial selection, migration, and genetic drift function at A19421G locus.

Table 1
Genotype frequencies and genetic diversity parameter at the bovine *AMPD1* gene

SNP	Genotypic frequencies			Allelic frequencies		χ^2 (HWE)	Diversity parameter			
							H_o	H_e	N_e	PIC
T19416C	TT	TC	CC	T	C					
	0.1860 (40/215)	0.4791 (103/215)	0.3349 (72/215)	0.4258	0.5744	P>0.05	0.5111	0.4889	1.9557	0.3694
A19421G	AA	AG	GG	A	G					
	0.6186 (133/215)	0.3814 (82/215	0.0000 (0/215)	0.8093	0.1907	P<0.05	0.6913	0.3087	1.4465	0.261

χ^2 (HWE): Hardy-Weinberg equilibrium χ^2 value, H_o: gene homozygosity, H_e: gene heterozygosity, N_e: effective allele numbers, PIC: polymorphism information content.

Association of the five diplotypes with growth and carcass traits in Qinchuan cattle breed

The association of the five diplotypes in *AMPD1* gene with growth and carcass traits (body length, body height, hip width, slaughter weight, carcass weight, and dressing percentage) in Qinchuan cattle (n=215) were analyzed (Table 2). Individuals with BC was significantly associated with carcass weight (P=0.049, P<0.05; n=56). The animals with BC had greater body length compared with AA (P=0.035, P<0.05; n=40) and CD (P=0.014, P<0.05; n=26) were observed, and the BC had greater slaughter weight compared with genotypes CD (P=0.021, P<0.05; n=26); the BC cattle had greater carcass weight than AA (P=0.045, P<0.05; n=40), CC (P=0.015, P<0.05; n=46) and CD (P=0.016, P<0.05; n=26), the BC diplotype had greater dressing percentage than AA (P=0.021, P<0.05; n=40). Other growth and carcass traits in the records had no significant association with diplotype studied. Therefore, the presence of two novel mutations in *AMPD1* gene might candidate gene that affects growth and carcass traits in Qinchuan cattle.

The previous studies showed that a few SNPs were detected in the *AMPD1* gene. Six SNPs were found in animals representing three commercial breeds (Yorkshire, Landrace, and Duroc) and three Chinese breeds (Meishan, Tongcheng & Qingping) of pigs. Three of the 6 mutations appeared in intronic regions, 1 in exon 11 and 2 in exon 12. The SNP (T426C)

in the coding region of exon 12 was a synonymous mutation. Association analysis revealed that a SNP (T426C) in the coding region of exon 12 (GenBank acc. no.: EU 606355) of the *AMPD1* gene was significantly associated with loin muscle area trait ($P<0.01$), loin muscle height ($P<0.01$) and average backfat thickness ($P<0.05$) (Wang *et al*. 2008). Walling and Cepica studies reported that the porcine *AMPD1* maps within known QTL (quantitative trait locus) with effects on carcass traits such as carcass weight, loin and neck meat weight, loin muscle area, shoulder meat weight, ham meat weight, chops weight. A new mutation was found in exon 5 (G468T). The G468T transversion is dysfunctional and further indicate that *AMPD1* alleles harboring this mutation contribute to the high incidence of partial and complete myoadenylate deaminase deficiency in the Caucasian population (Gross *et al*. 2002).

Both mutations (g. 19416T>C and g. 19421A>G) in bovine *AMPD1* were silent mutations, which can not result in the change of amino acid. But recently there were some reports about the effects of the silent mutations on the gene function and phenotype (Komar *et al*. 2007). A silent polymorphism in the MDR1 gene resulted in substrate specificity change (Kimchi *et al*. 2007). A silent mutation of goat POU1F1 gene had been found to associate with milk yield and birth weight (Lan *et al*. 2007). So, it's an interesting work to find out the mechanism for the association between these silent mutations and the growth and carcass traits in Qinchuan beef cattle.

Table 2
Effects (*P*-value) of polymorphism of the *AMPD1* gene on bovine growth and carcass traits

Traits	Diplotypes (Mean±SE)					*P*-value
	AA (n=40)	AC (n=47)	BC (n=56)	CC (n=46)	CD (n=26)	
BH, cm	139.3077 ± 1.5519	138.7143 ± 1.2211	141.1333 ± 1.0216	139.8947 ± 1.2837	139.0769 ± 1.5519	0.5916
BL, cm	148.7692 ± 1.7650^{a}	150.0476 ± 1.3887^{ab}	153.3000 ± 1.1619^{b}	151.7368 ± 1.4599^{ab}	148.0000 ± 1.7650^{a}	0.0666
HW, cm	46.8462 ± 1.0451	46.0714 ± 0.8222	46.8333 ± 0.6879	46.8684 ± 0.8644	48.6153 ± 1.0450	0.4500
SW, kg	491.1538 ± 17.0453^{ab}	499.8095 ± 13.4111^{ab}	509.4000 ± 11.2206^{a}	479.0526 ± 14.0993^{ab}	461.3077 ± 17.0453^{b}	0.1552
CW, kg	257.0308 ± 9.9501^{a}	266.6952 ± 7.8287^{ab}	281.2400 ± 6.5500^{b}	255.1263 ± 8.2304^{a}	252.0462 ± 9.9501^{a}	0.0491
DP, %	52.3046 ± 1.1444^{a}	53.5028 ± 0.9004^{ab}	55.5149 ± 0.7533^{b}	53.1175 ± 0.9466^{ab}	54.5957 ± 1.1443^{ab}	0.1140

SE: standard error of means, BH: body height, BL: body length, HW: hip width, SW: slaughter weight, CW: carcass weight, DP: dressing percentage
Values with different superscripts within the same line differ significantly at $P<0.05$.

The objectives of the present study were to identify sequence variation in bovine *AMPD1* gene and to evaluate associations between the polymorphisms and growth and carcass traits in Qinchuan cattle breed.

In summary, the present study reveals that the polymorphism of the bovine *AMPD1* gene is significantly associated with the body length, slaughter weight, carcass weight and dressing percentage, and no significant association with body height in Qinchuan cattle. The genotype BC tends to be better than those with the other genotypes in growth and carcass traits performance. Therefore, the presence of two SNPs of *AMPD1* gene might

influence growth and carcass traits in Qinchuan population. Furthermore, this study will be contributed to geneticists and breeders as a molecular marker for better performance in the bovine industry.

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