A novel 18-bp deletion mutation of the AMPD1 gene affects carcass traits in Qinchuan cattle

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Abstract The objectives of the present study were focused on detecting deletion mutation in bovine AMPD1 gene, and analyzing its effect on body measurement and carcass traits in Qinchuan cattle by using DNA sequencing and agarose electrophoresis methods. The 198-bp PCR products of AMPD1 gene exhibited three genotypes and two alleles were revealed: A and B. The frequencies of genotype AA/AB/BB in Qinchuan populations was 0.7163, 0.2233 and 0.0605. The $\chi^2$-test analysis demonstrated that the breed was not in agreement with Hardy–Weinberg equilibrium ($P < 0.05$). The association of the 18-bp deletion mutation of AMPD1 gene with body measurement and carcass traits of Qinchuan cattle were analyzed. The cattle with AA genotype had slaughter weight and carcass weight than those with genotype AB ($P < 0.01$ or $P < 0.05$). These results suggest that the 18-bp deletion mutation may influence the carcass traits in Qinchuan cattle.

Keywords AMPD1 gene · Deletion mutation · Carcass traits · Qinchuan cattle

Introduction

Adenosine monophosphate deaminase (AMPD) is a complex allosteric enzyme encoded by a multigene family in mammals. Multiple isoforms have been isolated from different human and animal tissues and are named after the source of their purification [1]. The AMPD enzyme is an important regulator of cellular energy metabolism through its participation in purine nucleotide catabolism [2]. 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 [3–6]. It is likely that the three AMPD genes arose from duplication of a common primordial gene [7], 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 SLSTDDP believed to be the catalytic center of the enzyme [8, 9]. 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 [10]), 47–128 (AMPD2 [11]), and 7–9 (AMPD3 [5]) 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 [12].

The AMPD1 gene is highly expressed in skeletal muscle, and is the rate-limiting step in the purine nucleotide cycle, allowing repletion of ATP stores. AMP deaminase (AMPD; EC 3.5.4.6) catalyzes the hydrolytic deamination of adenosine-50-monophosphate (AMP) to inosine-50-monophosphate (IMP) and ammonium ion. It is expressed predominantly in skeletal muscle and the abundance of this transcript increases during muscle development in vivo and

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during myocyte differentiation in vitro [13]. The skeletal muscle fiber type may be influenced by an \( \text{AMPD1} \)-dependent transcriptional pathway, and the knowledge of modulation of \( \text{AMPD1} \) gene will contribute to the understanding of muscle development.

In human, the \( \text{AMPD1} \) gene is specific to isoform M, and has been cloned and localized to the short arm of chromosome 1 in the region p13–p21 [3]. This gene is expressed predominantly in skeletal muscle and isoform M is relatively more abundant in type II myofibers. \( \text{Cis} \) acting elements located in proximal sequence upstream from the \( \text{AMPD1} \) transcriptional start site are required for skeletal myocyte expression of the gene.

In porcine, the \( \text{AMPD1} \) gene was mapped to SSC 4q1.6–q2.3 [14]. Previous studies indicated that the genes involved in energy metabolism were closely related to growth and carcass traits of \( \text{Sus scrofa} \). The \( \text{AMPD1} \) gene might be a candidate gene of meat production trait and provides useful information for further studies on its roles in porcine skeletal muscle [15], etc. Therefore, we focus on bovine \( \text{AMPD1} \) gene, which could be candidate genes of bovine body measurement and carcass traits.

At present, no study has revealed any genetic information relevant to bovine \( \text{AMPD1} \) gene. The goal of our study was to identify sequence variation of \( \text{AMPD1} \) gene in Qinchuan cattle breed, and to analyze the relationship between gene variation and body measurement and carcass traits, which will benefit for cattle breeding and genetics.

**Material and methods**

**Animal source**

A total of 215 cattle 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. The records of body measurement traits (body length, withers 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.

**DNA preparation and primer design**

Genomic DNA of 215 cattle were isolated from 2% heparin-treated blood samples and stored at \(-80^\circ\text{C}\), following the standard procedures [16]. Primers used to amplify bovine \( \text{AMPD1} \) gene intron 8 locus were designed from a published gene sequence (GenBank accession number: NC_007301). The sequences of the primers: 1F: 5’-CAA ACA CTC CCT TCT CA-3’ (nt 16040–16056); 1R: 5’-TAG TGC CTG ACC CAA GT-3’ (nt 16542–16558); 2R: 5’-ATG CTT ATG AGA GCT GGC-3’ (nt 16220–16237). The size of expected PCR products was 519 bp and 198 bp, containing the whole exon 9 and partial of the intron 8 and intron 9 regions.

**DNA sequencing and agarose electrophoresis analysis**

PCR was performed in a 25 \( \mu \text{l} \) of reaction volume, containing 50–100 ng genomic DNA, 10 pM of each primer, 1× buffer (including 1.5 mM MgCl\(_2\), 200 \( \mu \text{M} \) dNTPs and 1.5 units of \( \text{Taq} \) DNA polymerase (MBI). The PCR protocol for \( \text{AMPD1}-1\text{F}-1\text{R} \) was 3 min at 95°C, 35 cycles of 94°C for 30 s, 56°C annealing for 40 s, 72°C for 40 s, with a final extension at 72°C for 10 min. The PCR protocol for \( \text{AMPD1}-1\text{F}-2\text{R} \) was 3 min at 95°C, 35 cycles of 94°C for 30 s, 55°C annealing for 40 s, 72°C for 30 s, with a final extension at 72°C for 10 min.

The products of 519-bp fragment were purified by using the DNA Fragment Purification Kit (BIODEV Corp., Beijing, 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).

The 198-bp fragment of the \( \text{AMPD1}-1\text{F}-2\text{R} \) was designed to detect for these polymorphisms. PCR products were electrophoresed on 3% agarose gels with 1× TBE buffer, containing 200 ng/ml ethidium bromide. A 7 \( \mu \text{l} \) aliquot of PCR products was added to 1.5 \( \mu \text{l} \) of loading dye (0.025% bromophenol blue, 0.025% xylene cyanol, 40% (w/v) sucrose) and the gels were run at a constant voltage (100 V) for 50 min.

**Statistical analysis**

Gene frequencies were determined for each breed by direct counting. \( \chi^2 \) tests were used to determine if the individual variant was in Hardy–Weinberg equilibrium. The genotype and allele frequency distributions were compared by Chi-square test. Levels of genetic variability were estimated with the unbiased expected homozygosity (Ho) and heterozygosity (He), the effective allele numbers (Ne), the polymorphic information content (PIC). The formulas were as follows:

\[
H_o = \frac{n}{\sum_{i=1}^{n} P_i^2} = 1 - \sum_{i=1}^{n} P_i^2 Ne = 1 - \sum_{i=1}^{n} P_i^2 PIC
\]

\[
= 1 - \sum_{i=1}^{m} P_i^2 - \sum_{i=1}^{m} \sum_{j=i+1}^{m} 2P_i^2 P_j^2
\]
"Pi" is the frequency of the i allele, "n" is the number of alleles.

The traits were compared between the genotypes. The relationship between genotypes of the AMPD1 gene and body measurement and carcass traits were analysed according to one-way analysis [17, 18], using the following model:

\[ Y_{ijkl} = \mu + BF_i + M_j + G_k + e_{ijkl} \]

where \( Y_{ijkl} \) = observed value; \( \mu \) = overall mean for each trait; \( BF_i \) = fixed effect of \( i \)th breed and farm; \( M_j \) = fixed effect of \( j \)th month of slaughtering; \( G_k \) = fixed effect of \( k \)th single SNP marker genotype; \( e_{ijkl} \) = random error.

**Results and discussion**

DNA sequencing and genotype distribution

Intron 8 region of cattle AMPD1 gene demonstrated polymorphic patterns in Qinchuan populations by DNA sequencing method. In this paper, the 198-bp fragment of the AMPD1-1F-2R was showed polymorphism in the studied population; the genotypes (AA, AB and BB) were detected at AMPD1 gene intron 8 locus. The 3.0% agarose gel electrophoresis was then used to verify the polymorphisms (Fig. 1). The DNA sequence was deposited in GenBank database (GenBank accession number: GQ153529). The comparison between nucleotide sequence of bovine AMPD1 gene (GenBank accession number: NC_007307) and the GQ153529 sequence revealed a 18-bp deletion. According to the mutation nomenclature and proposals [19], the 18-bp deletion mutation was described as g.16164–16181 del TTC CCC TCA TAC CAC GCC (Fig. 2).

**Analysis of polymorphism of the AMPD1 gene in Qinchuan cattle breed**

The \( \chi^2 \)-test showed that the genotype distributions of Qinchuan breed in disagreement with at Hardy–Weinberg equilibrium \((P < 0.05)\), which showed that there was not a dynamic equilibrium even in artificial selection, migration, and genetic drift function. The frequencies of genotype AA/AB/BB in Qinchuan populations was 0.7163, 0.2233 and 0.0605. The frequencies of allele A/B of Qinchuan populations were 0.8279/0.1721. In present populations, the population genetic parameters of homozygosity, heterozygosity, effective allele numbers and PIC (Polymorphism Information Content) were 0.8279, 0.1721, 1.3985 and 0.2444 (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 intermediate genetic diversity, this reflected that there was not a very high genetic diversity within Chinese bovine AMPD1 gene in the analyzed population.

**Association of polymorphism of the 18-bp deletion with body measurement and carcass traits in Qinchuan cattle breed**

The association of 18-bp deletion mutation in AMPD1 gene with body measurement and carcass traits (body length, withers height, and hip width, slaughter weight, carcass weight, dressing percentage) in Qinchuan cattle (\( N = 215 \)) were analyzed (Table 2). The animals with genotype AA greater slaughter weight compared with genotype AB \((P < 0.01)\) was observed and the genotypes AA had greater carcass weight compared with genotypes AB \((P < 0.05)\). Other growth traits in the records had no significant association with genotypes studied. Therefore, the presence of 18-bp deletion mutation in AMPD1 gene might candidate gene that affects body measurement and carcass traits in Qinchuan cattle.

Only a few polymorphisms were detected so far in the AMPD1 gene. Six polymorphic sites were reported by Wang et al. [15]. These single nucleotide polymorphisms (SNPs) were found in animals representing three introduced commercial breeds (Yorkshire, Landrace, and Duroc) and three Chinese breeds (Meishan, Tongcheng and Qingping) of pigs. Three of the six mutations appeared in intronic regions, one in exon 11 and two 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 accession number: 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 ($P < 0.05$). Several previous 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 [20, 21]. 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 [22].

Up to now, the research about any genetic information relevant to bovine AMPD1 gene was not reported. The objectives of the present study were to identify sequence variation in bovine AMPD1 gene and to evaluate associations between the polymorphisms and body measurement and carcass traits in Qinchuan cattle population.

In this study, the associations between the 18-bp deletion mutation of AMPD1 gene and the body measurement and carcass traits performance showed that the genotype had significant effect on slaughter weight and carcass weight in Qinchuan population, some of those with better performance of AA genotype could be used for the breeding of new breeds of beef cattle in China. Therefore, the presence of 18-bp deletion mutation of AMPD1 gene might negatively influence body measurement and carcass traits.

### Table 1 Genetic diversity at AMPD1 gene intron 8 locus in Qinchuan cattle

<table>
<thead>
<tr>
<th>Genotypic frequencies</th>
<th>Allelic frequencies</th>
<th>$\chi^2$ (HWE)</th>
<th>$Ho$</th>
<th>$He$</th>
<th>$Ne$</th>
<th>PIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA (0.7163 (154/215))</td>
<td>0.8279</td>
<td>$P &lt; 0.05$</td>
<td>0.8279</td>
<td>0.1721</td>
<td>1.3985</td>
<td>0.2444</td>
</tr>
<tr>
<td>AB (0.2233 (48/215))</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>BB (0.0605 (13/215))</td>
<td>0.1721</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

$\chi^2$ (HWE) Hardy–Weinberg equilibrium, $Ho$ gene homozygosity, $He$ gene heterozygosity, $Ne$ effective allele numbers, PIC polymorphism information content

### Table 2 Effects ($P$-value) of polymorphism of 18-bp deletion within the AMPD1 gene on bovine body measurement and carcass traits

<table>
<thead>
<tr>
<th>Traits</th>
<th>Genotypes</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA (n = 154) (mean ± SE)</td>
<td></td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>140.242 ± 0.684</td>
<td>0.556</td>
</tr>
<tr>
<td>Body length (cm)</td>
<td>151.530 ± 0.795</td>
<td>0.188</td>
</tr>
<tr>
<td>Hip width (cm)</td>
<td>47.379 ± 0.456</td>
<td>0.124</td>
</tr>
<tr>
<td>Slaughter weight (kg)</td>
<td>505.515 ± 7.326 $^A$</td>
<td>0.006</td>
</tr>
<tr>
<td>Carcass weight (kg)</td>
<td>271.658 ± 4.582 $^a$</td>
<td>0.032</td>
</tr>
<tr>
<td>Dressing percentage (%)</td>
<td>53.779 ± 0.513</td>
<td>0.191</td>
</tr>
<tr>
<td></td>
<td>AB (n = 48) (mean ± SE)</td>
<td></td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>139.000 ± 1.134</td>
<td></td>
</tr>
<tr>
<td>Body length (cm)</td>
<td>150.625 ± 1.318</td>
<td></td>
</tr>
<tr>
<td>Hip width (cm)</td>
<td>46.187 ± 0.756</td>
<td></td>
</tr>
<tr>
<td>Slaughter weight (kg)</td>
<td>462.042 ± 12.148 $^B$</td>
<td></td>
</tr>
<tr>
<td>Carcass weight (kg)</td>
<td>254.283 ± 7.362 $^b$</td>
<td></td>
</tr>
<tr>
<td>Dressing percentage (%)</td>
<td>55.155 ± 0.851</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BB (n = 13) (mean ± SE)</td>
<td></td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>138.714 ± 2.100</td>
<td></td>
</tr>
<tr>
<td>Body length (cm)</td>
<td>146.857 ± 2.440</td>
<td></td>
</tr>
<tr>
<td>Hip width (cm)</td>
<td>44.786 ± 1.400</td>
<td></td>
</tr>
<tr>
<td>Slaughter weight (kg)</td>
<td>465.000 ± 22.494 $^{AB}$</td>
<td></td>
</tr>
<tr>
<td>Carcass weight (kg)</td>
<td>242.286 ± 13.631 $^b$</td>
<td></td>
</tr>
<tr>
<td>Dressing percentage (%)</td>
<td>52.177 ± 1.576</td>
<td></td>
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</tbody>
</table>

Note: Values with different superscripts within the same line differ significantly at $P < 0.01$ (A, B) and $P < 0.05$ (a, b), SE standard error of means.

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Fig. 2 The sequencing and sequence comparison results of AMPD1 gene in bovine. (AA) The sequencing results of AMPD1-AA genotype in bovine. (AB) The sequencing results of AMPD1-AB genotype in bovine. (BB) The sequencing results of AMPD1-BB genotype in bovine.
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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References