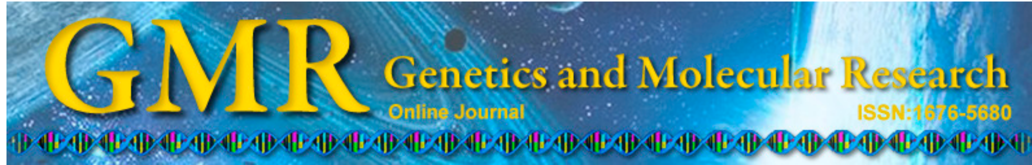


# DNA sequence polymorphism within the bovine adenosine monophosphate deaminase 1 (AMPD1) is associated with production traits in Chinese cattle

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**ABSTRACT.** The objectives of the present study were to detect an 18-bp deletion mutation in the bovine adenosine monophosphate deaminase 1 (*AMPD1*) gene and analyze its effect on growth traits in 2 Chinese cattle breeds using DNA sequencing and agarose electrophoresis. The five 19-bp polymerase chain reaction products of the AMPD1 gene exhibited 3 genotypes and 2 alleles: WW: homozygote genotype (wild-type); DD: homozygote genotype (mutant-type); WD: heterozygote genotype. Frequencies of the W allele varied from 66.15-

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70.35%. The associations between the 18-bp deletion mutation in the *AMPD1* gene with production traits in 226 Jia-Xian red cattle was analyzed. The animals with genotype WW showed significantly higher heart girth and body weight than those with genotypes WD and DD at 24 months (P < 0.01). Our results indicate that the deletion mutation in the *AMPD1* gene is associated with production traits, and may be used for marker-assisted selection in beef cattle breeding programs.

**Key words:** *AMPD1* gene; Cattle; Deletion mutation; Production traits

## 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 their source of purification. The AMPD enzyme regulates cellular energy metabolism by participating in purine nucleotide catabolism (Zimmerman, 1992). Subsequent cloning of 3 human genes revealed the molecular basis of 4 different isoforms: AMPD1, isoforms M, muscle; AMPD2, isoforms L, liver; AMPD3, isoforms E1 and E2, erythrocyte (Bausch-Jurken et al., 1992; Mahnke-Zizelman et al., 1996). The 3 AMPD polypeptides share a similar 550-amino acid C-terminal end (62-70% identical) containing the motif SLSTDDP, which is thought to be the catalytic center of the enzyme (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 humans, the *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 (Sabina et al., 1990). This gene is expressed predominantly in skeletal muscle and is relatively more abundant in type II myofibers. *Cis*-acting elements located in proximal sequence upstream from the *AMPD1* transcriptional start site are required for skeletal myocyte gene expression. AMPD1 is preferentially expressed at high levels in type II skeletal muscle, where it influences the levels of inorganic phosphate, AMP, ADP, and phosphocreatine (Coley et al., 2012). Patients with an inherited defect in AMPD1 expression often have significantly decreased muscle performance, suggesting that the purine nucleotide catabolic pathway plays a role in short-term energy production (Fischer et al., 2007; Norman et al., 2008). The *AMPD1* gene is highly expressed in skeletal muscle, and is involved in the rate-limiting step of the purine nucleotide cycle, allowing repletion of ATP stores. AMP deaminase (AMPD; EC 3.5.4.6) catalyzes the hydrolytic deamination of adenosine monophosphate (AMP) to inosine 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 during myocyte differentiation *in vitro* (Sabina et al., 1989). The skeletal muscle fiber type may be influenced by an *AMPD1*-dependent transcriptional pathway, and studies examining modulation of the *AMPD1* gene will contribute to the understanding of muscle development.

In porcine, the *AMPD1* gene was found to be involved in energy metabolism closely related to growth and carcass traits (Wang et al., 2008). The *AMPD1* gene may be a candidate gene for production trait and provides useful information for further studies on its roles in bovine skeletal muscle. Therefore, we focused on the bovine *AMPD1* gene, which may be candidate genes of bovine production traits.

The purpose of this study was to identify an 18-bp deletion mutation in the bovine *ZBED6* gene in 2 Chinese cattle populations. We also conducted association analysis to increase the understanding of the role of *ZBED6* in the variation of production traits in cattle, which may be useful for animal breeding and genetic programs.

## MATERIAL AND METHODS

### Animal source, DNA preparation and growth data

Genomic DNA samples were obtained from 356 cows belonging to 2 cattle breeds: Jia-Xian red cattle (JX, N = 226), and Nan-Yang yellow cattle (NY, N = 130). These 2 cattle breeds represent the main breeds of China and are typically reared in the Province of Henan. The Jiaxian animals were from the breeding farm of Jia-Xian Cattle (Jia-Xian County, Henan Province, China); the Nanyang animals were from the Nan-Yang Cattle breeding center (Nan-Yang City, Henan Province, China).

Genomic DNA from 356 cattle were isolated from 2% heparin-treated blood samples and stored at -80°C, following the standard procedures (Sambrook and Russell, 2002).

The 226 cows of the JX breed used for the association study were from a common ancestor, and pedigrees of core breeding population animals were traced back 3 generations. The animals were weaned at an average of 6 months age and raised from weaning to slaughter on a corn silage diet. The growth traits (withers height, body length, heart girth, hucklebone width, and body weight) at 24 months were measured following the method described by Gilbert et al. (1993). All experimental procedures were performed according to authorization granted by the Chinese Ministry of Agriculture.

### Primer design and polymerase chain reaction (PCR) amplification

Primers used to amplify the bovine *AMPD1* gene intron 8 locus were designed from a published gene sequence (GenBank accession No. NC_007301). The sequences of the primers: F: 5'-CAA ACA CTC CCT TCT CA-3' (nucleotides 16040-16056); R: 5'-TAG TGC CTG ACC CAA GT-3' (nucleotides 16542-16558). The sizes of expected PCR products were 5 (19 bp, containing all of exon 9 and part of the intron 8 and intron 9 regions (Figure 1).

The 25-mL volume PCR amplification contained 50-100 ng genomic DNA, 10 pM of each primer, 1X buffer (including 1.5 mM $MgCl_2$), 200 mM dNTPs, and 1.5 U *Taq* DNA polymerase (MBI, Vilnius, Lithuania). The PCR protocol for *AMPD1*-F-R was 3 min at 95°C, 35 cycles at 94°C for 30 s, 56°C annealing for 40 s, 72°C for 40 s, and a final extension at 72°C for 10 min.

### DNA sequencing and agarose electrophoresis analysis

After polymorphism detection, the PCR products of different electrophoresis patterns were purified using the DNA Fragment Purification Kit (BIODEV Corp., Beijing, China) and sequenced in both directions (Beijing Aolaibo Biotechnology, China; Applied Biosystems 3730xl DNA Sequencer, Foster city, CA, USA); sequences were analyzed using the BioXM software (Version 2.6).

The 18-bp deletion of the bovine *AMPD1* gene was detected. PCR products were

electrophoresed on 3% agarose gels with 1X TBE buffer, containing 200 ng/mL ethidium bromide. A 7-mL aliquot from the PCR was added to 1.5 mL 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.

CAAACACTCCCTTCTCAAAGCTTTAACCATGTGGGCTTACCATGCTGACAGAAAGAACCCATGCACAATTTCAAGGTTCTCTCATTGTCAGCTATCCTG
**>>>>>Primer F>>>>>>**
TATTCCATTCTTGGAGCCCGAGCCCTTCCCCTCATACCACGCCCTGGTTCCTTGCAGTCATTCCCTAACTCTCTGTGTCCACGCAGCTCTCATAAGCATA
****18-bp deletion region****
CACTTGTGTTCTGTTTCCTGATGCAGGGACGTCAGACTTTCCAGCGTTTTGATAAATTCAATGACAAATACAATCCCGTAGGAGCAAGTGAGCTACGGGA
CCTCTACCTGAAGACAGACAATTACATTAATGGGGAATATTTTGCCACTATCATCAAGGTGAGGGGGAACCAGCCAGATCTACGGTACCTGGCCTGATTC
TTAGGGAGGGGGAAGAAGAAAGGGAGGGAAGGAAGAAGGAAGCGGGGAAGGAAGCAGGGGAGAAGAAGGGGAAGGAGAGAGGAGGCG
GAGGGAGAGTGCTGTCACTTGGGTCAGGCACTA
<<<<<<**Primer R**<<<<<

**Figure 1.** Schematic representation of the 519-bp fragment of the AMPD1 gene (modified output of the bovine AMPD1 gene). Nucleotide sequences for a 519-bp AMPD1 fragment (upper strands) and its bisulfite-converted version (lower strands). Primers sequences are marked with arrows**.** Squared nucleotides contain 18-bp deletion region ("TTCCCCTCATACCACGCC") for 3% agarose gel electrophoretic analyses.

## Statistical methods

Gene frequencies were determined for each breed by direct counting. A $\chi^2$ test was applied to assess statistical significance using the SPSS software (Version 16.0, SPSS, Inc., Chicago, IL, USA). Population genetic indexes, such as gene heterozygosity ($H_E$), effective allele numbers ($N_E$), and polymorphism information content (PIC) were calculated according to Nei's methods (Nei and Roychoudhurg, 1974). The formulas were as follows:

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

where "$P_i$" is the frequency of the i allele, "n" is the number of alleles.

Statistical analysis was performed on records of growth traits in JX cattle. The SPSS software was used to analyze the relationship between the genotypes and traits in cattle. The linear model included fixed effects of age and genotype (Boldman et al., 1995; Zhao et al., 2004). The linear model was as follows:

$$Y_{ijk} = \mu + A_i + G_j + E_{ijk}, \qquad \text{(Equation 2)}$$

where $Y_{ijk}$ = the trait measured on each of the $ijk^{th}$ animal; $\mu$ = the overall population mean; $A_i$ = the fixed effect due to the $i^{th}$ age; $G_j$ = the fixed effect associated with $j^{th}$ genotype; $E_{ijk}$ = the random error. The least square mean estimates with standard errors for the 2 *AMPD1* gene genotypes and growth traits were used.

# RESULTS

## DNA sequencing and genotype distribution

The bovine *AMPD1* gene is located on chromosome 3 and has been revealed to contain 12 exons. To better understand the detailed genetic variation in the bovine *AMPD1* gene, DNA sequencing was used. The comparison between nucleotide sequence of bovine *AMPD1* gene (GenBank accession No. AC_000160.1) revealed an 18-bp deletion in the intron 8 region of the bovine *AMPD1* gene (Figure 2).

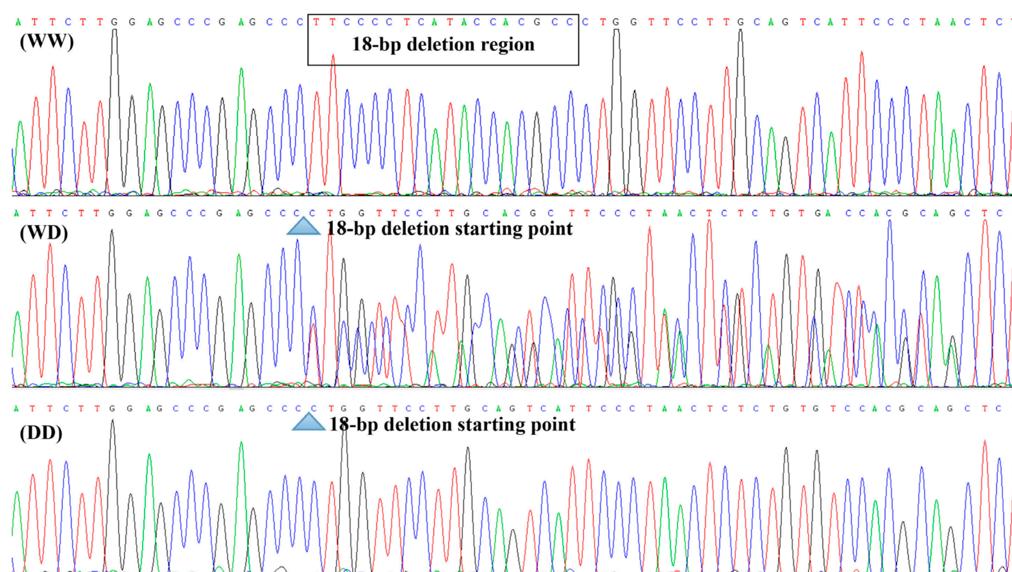


**Figure 2.** Sequencing and sequence comparison results of the bovine AMPD1 gene. WW = the sequencing results of AMPD1-WW genotype in cattle; WD = the sequencing results of AMPD1-WD genotype in cattle; DD = the sequencing results of AMPD1-DD genotype in cattle.

In this study, the 5 (19-bp) fragment of the *AMPD1* gene showed 2 genotypes in the population studied. The fragment lengths of 5 (19 bp for genotype WW, 501 bp for genotype DD, and 5 (19 and 501 bp) for genotype WD were detected at *AMPD1* gene intron 8. We conducted 3.0% agarose gel electrophoresis to verify the polymorphisms (Figure 3).

## Diversity analyses

Frequencies of the *AMPD1*-W allele in the populations analyzed were 70.35 and 66.15% for JX and NY, respectively. The frequency of allele W was higher in the 2 breeds. The $\chi^2$ test showed that the genotype distributions within the JX and NY breeds were in Hardy-Weinberg equilibrium ($P > 0.05$; Table 1), indicating that there was dynamic equilibrium even in artificial selection, migration, and genetic drift function.

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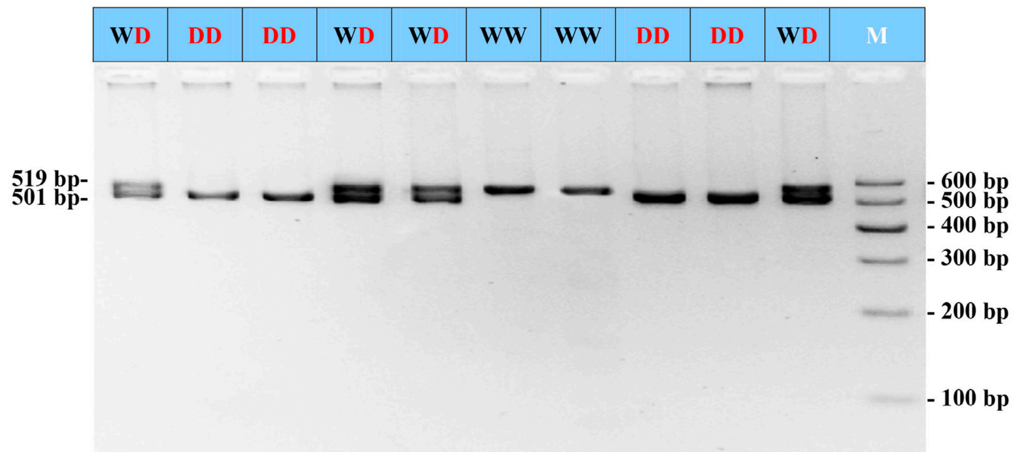


**Figure 3.** Agarose gel (3%) electrophoretic patterns of the DNA region containing the intron 8 of the bovine AMPD1 gene. M: marker I (600; 500; 400; 300; 200; 100 bp); WW (519 bp); WD (519 and 501 bp); DD (501 bp).

Genetic indices ($H_E$, $N_E$, and PIC) in these 4 Chinese cattle populations are presented in Table 1. The values of the observed gene heterozygosity approached 0.5 in the 2 cattle breeds. The maximum and minimum PIC values were 0.3301 and 0.3475, respectively, and were classified as low (PIC value < 0.25, low polymorphism; 0.25 < PIC value < 0.5, intermediate polymorphism; and PIC value > 0.5, high polymorphism). The 2 Chinese cattle breeds showed low genetic diversity. This reflected that genetic diversity was not high within the Chinese bovine *AMPD1* gene in the populations analyzed.

**Table 1.** Genotypic and allelic frequencies (%) and diversity parameter of the bovine *AMPD1* gene.

| Breed (number) | Genotype number/ Genotypic frequency (%) | | | Allele/Allelic frequency (%) | | $\chi^2$ | $H_E$ (Gene heterozygosity) | $N_E$ | PIC |
|---|---|---|---|---|---|---|---|---|---|
| | WW | WD | DD | W | D | (HWE) | | | |
| Jia-Xian red cattle (226) | 118/52.22 | 82/36.28 | 26/11.50 | 70.35 | 29.65 | 3.8310* | 0.5828 | 1.7156 | 0.3301 |
| Nan-Yang yellow cattle (130) | 61/46.92 | 50/38.46 | 19/14.62 | 66.15 | 33.85 | 2.5889* | 0.5522 | 1.8109 | 0.3475 |

WW = homozygote genotype (wild-type); DD = homozygote genotype (mutant-type); WD = heterozygote genotype. $\chi^2$ (HWE) = Hardy-Weinberg equilibrium $\chi^2$ value. *Hardy-Weinberg equilibrium (P > 0.05), Hardy-Weinberg disequilibrium (P < 0.05). $N_E$ = effective allele numbers. PIC = polymorphism information content.

## Associations between different genotypes and production traits in JX cattle

We examined the association between polymorphisms in the *AMPD1* gene and production traits in the JX breed. Animals with the WW genotype showed higher heart girth and body weight than those with genotypes WD and DD (P < 0.01) at 24 months (Table 2). The remaining records of production traits showed no significant association with the genotype (P > 0.05). Therefore, the presence of the 18-bp deletion mutation in the *AMPD1* gene may be a candidate that affects production traits in JX cattle.

**Table 2.** Associations between 18-bp deletion of the *AMPD1* gene and phenotypic traits in Jia-Xian red cattle (means ± SE).

| Phenotypic traits | Genotypes (number) | | | P value |
|---|---|---|---|---|
| | WW (118) | WD (82) | DD (26) | |
| Withers height (cm) | 123.571 ± 1.764 | 122.34 ± 0.641 | 122.562 ± 1.167 | 0.805 |
| Body length (cm) | 139.286 ± 2.638 | 135.585 ± 0.959 | 132.062 ± 1.745 | 0.062 |
| Heart girth (cm) | $172.786 \pm 3.034^{A}$ | $165.547 \pm 1.102^{B}$ | $159.219 \pm 2.007^{C}$ | 0.001 (P < 0.01) |
| Hucklebone width (cm) | 26.929 ± 0.951 | 26.047 ± 0.346 | 25.687 ± 0.629 | 0.556 |
| Body weight (kg) | $384.824 \pm 18.306^{A}$ | $346.802 \pm 6.653^{B}$ | $309.931 \pm 12.108^{C}$ | 0.003 (P < 0.01) |

Data are reported as least square means ± standard errors (means ± SE). Values with different superscript letters within the same column differ significantly at P < 0.01.

## DISCUSSION

Only a few polymorphisms have been detected in the *AMPD1* gene. In humans, this has been confounded by a common mutant allele in *AMPD1* in Caucasians known as C34T. This allele introduces a premature stop codon in the second exon of AMPD1, but few studies have examined the 2 major splice variants in *AMPD1*, the second of which drops exon 2 without affecting enzyme activity (Morisaki et al., 1993). Several human studies have assumed that individuals who are homozygous for C34T possess 0% enzyme activity, resulting in potentially erroneous conclusions. Importantly, 2 different mutations in *AMPD1* (R388W and R425H) have been identified in Japanese patients with very low AMPD1 enzyme activity and symptoms of myalgia, weakness, and exercise intolerance (Morisaki et al., 2000).

Six single nucleotide polymorphisms were found in animals, representing 3 introduced commercial breeds (Yorkshire, Landrace, and Duroc) and 3 Chinese breeds (Meishan, Tongcheng, and Qingping) of pigs (Wang et al., 2008). Three of the 6 mutations appeared in intronic regions, 1 in exon 11, and 2 in exon 12. The single nucleotide polymorphism (T426C) in the coding region of exon 12 was a synonymous mutation. Association analysis revealed that a single nucleotide polymorphism (T426C) in the coding region of exon 12 of the *AMPD1* gene was significantly associated with loin muscle area (P < 0.01), loin muscle height (P < 0.01), and average back fat thickness (P < 0.05). Several previous studies reported that the porcine *AMPD1* maps within a known 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, and chop weight (Geldermann et al., 1999; Walling et al., 2000; Cepica et al., 2003). A new mutation was found in exon 5 (G468T); the G468T transversion is dysfunctional and further indicates 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). In cattle, the association between the 18-bp deletion mutation in the *AMPD1* gene and body measurement and carcass traits of Qinchuan cattle were analyzed, and the cattle with the AA genotype (wild-type) had slaughter weight and carcass weight greater than those with genotype AB (P < 0.01 or P < 0.05) (He et al., 2010).

## CONCLUSIONS

In this study, the association between the *AMPD1* gene and performance traits indicated that the genotype had a significant effect on heart girth and body weight in the JX population. Traits with better performance in the WW genotype can be used for the breeding

of beef cattle in China. However, further research and validation of the various allelic effects, functional mechanisms, and the bioactivity are needed in an independent sample prior to claiming that the *AMPD1* gene 18-bp deletion mutation or others can be used for marker-assisted selection in beef cattle.

## Conflicts of interest

The authors declare no conflict of interest.

## ACKNOWLEDGMENTS

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