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Back fat thickness and meat tenderness are associated with a 526 T→A mutation in the exon 1 promoter region of the MyF-5 gene in Chinese *Bos taurus*

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ABSTRACT. Qualitative trait loci (QTL) for growth and meat quality traits in cattle (*Bos taurus*) have been previously mapped to three chromosome regions, 0 to 30, 55 to 70, and 70 to 80 cM on chromosome 5. We evaluated the allele frequencies and gene-specific single nucleotide polymorphisms (SNPs) of bovine myogenic factor 5 (MyF-5) in the QTL regions and their associations with live weight and meat characteristics in indigenous Chinese cattle breeds. PCR-SSCP methodology showed a T>A mutation at 526 bp. Least square analysis revealed a significant association of this SNP with backfat thickness and meat tenderness ($P < 0.05$), while no significant association was found with live weight, loin eye height, loin eye area, rib area, or water holding capacity. Allele frequencies of MyF-5-A/B in the five breeds were 0.760/0.239, 0.752/0.247, 0.629/0.370, 0.715/0.284, and 0.750/0.250, for JiaXian red, Luxi, Nanyang, Qinchuan, and XiaNan

crossbreed, respectively. The genotype distributions for these alleles in two of the Chinese cattle breeds (Luxi and Qinchuan) were not in Hardy-Weinberg equilibrium ($P < 0.05$); while those for the other three breeds (JiaXian red, Nanyang, and XiaNan) were in agreement with Hardy-Weinberg equilibrium ($P > 0.05$). The genotypic frequencies among all five cattle breeds showed moderate diversity ($0.25 <$ polymorphism information content < 0.5). Based on our findings, we suggest that the MyF-5 gene influences back fat thickness and meat tenderness in Chinese *Bos taurus*. This SNP could be useful for marker-assisted selection for meat quality traits in these cattle.

Key words: Association; Cattle; Genotypic frequencies; Mutation; Myogenic factor 5; Meat quality

INTRODUCTION

Most of the quantitative attributes in animal production express continuous variations among *Bos taurus* cattle and thereafter their genetic nature is very complex. Generally, two types of procedures are used for the identification of the quantitative traits loci (QTL) in animals: 1) mapping of hypothetical genes by linkage analysis and 2) association analysis of candidate gene polymorphism on a trait of interest. QTL for meat quantity and meat quality traits in cattle have been identified by many researchers (Davis et al., 1998; Stone et al., 1999; Casas et al., 2000). Myogenic factor 5 (MyF-5) has also been well mapped for QTL, for birth weight, pre-weaning average daily gain, and average daily gain on cattle chromosome 5 (Li et al., 2002a,b). Thereafter, three chromosomal regions (0 to 30, 55 to 70, and 70 to 80 cM) were acknowledged that are significantly associated with growth and meat quality traits. Muscle formation is a multistep process involving commitment, proliferation, and specification during embryo growth to postnatal maturation and function (te Pas et al., 1999) and is controlled by myogenic determination (MyoD) gene family. MyoD consists of 4 family members: MyF-3 (MyoD) 1, MyF-4 or Myogenin (MyoG), MyF-5, and MyF-6 genes. The role of myogenic factors 5 and 6 is considered to be inherent for innovation and growth of straight muscle and for the sustainment of its physical appearance. Hence, they are believed to be candidate genes for growth and meat quality characteristics (Maak et al., 2006; Verner et al., 2007). A study by Shin and Chung (2007) on candidate genes offers the identification of SNPs in genes that most likely cause mutation in a phenotypic trait based on physiological and endocrinological evidence. The positional candidate genes and their association with meat quality traits provide an excellent opportunity for marker assisted selection (Khatib et al., 2007; Dario et al., 2009). The MyF-5 gene was mapped in a QTL position between 0 and 30 cM on BTA5 (Li et al., 2002b). Therefore, this gene is a strong candidate gene for meat quality traits in Chinese *B. taurus*. In cattle, the MyF-5 gene has a length of 3236 bp, with 3 exons and 2 introns (Gene Bank accession No. NC_007303). Polymorphism in the MyF-5 gene has been previously described in the literature to be associated with growth traits in Canadian cattle (Li et al., 2004); growth and average daily weight gain in Korean (Han woo) cattle (Chung and Kim, 2005); growth traits in Chinese (Qinchuan) cattle (Zhang et al., 2007), and growth and carcass traits in Korean (Han woo) cattle (Bhuiyuan et al., 2009). However, limited research has been car-

ried out regarding the relationship between the polymorphism of the MyF-5 gene and meat quality traits. Therefore, the objectives of this study were to determine the polymorphism of the MyF-5 gene, to evaluate the allelic and genotypic frequencies and also to determine the polymorphic information index in Chinese *B. taurus* cattle.

MATERIAL AND METHODS

Animals

A total of 544 adult animals namely JiaXian red (JXR = 115), Qinchuan (QC = 225), Luxi (LX = 93), Nanyang (NY = 77), and XiaNan (XN = 34) were selected from the dissimilar commercial breeding populations and were subjected to analysis of MyF-5 allelic frequencies. The animals were selected at the age of 2 years and were reared in the Provinces of Henan, Shaanxi, Shandong, Henan, and Henan, respectively. The association analysis of 440 meat quality traits was assessed as previously described (Wheeler et al., 1994), considering back fat thickness (BFT), loin eye height (LEH), loin eye area (LEA), live weight (LW), marbling (Mb), meat tenderness (MT), rib area (RA), and water holding capacity (WHC). Mb for meat quality grade was examined on a cross section of the loin muscle between the 12th and 13th rib and was scored from 1 to 5.

DNA extraction and PCR amplification

Five hundred and forty-four blood samples were obtained from five indigenous Chinese cattle breeds. DNA was extracted from leukocytes and assorted from acid citrate dextrose blood samples (ACD:blood, 1:6), treated with 2% heparin, and stored at -80°C, observing the standard method prescribed by Sambrook and Russell (2002). According to the NCBI database of the bovine MyF-5 gene (Gene-Bank accession No. NC_007303), one primer pair forward (5'-CCAACTATCCACCAGTAA-3') and reverse (5'-ACGACCAACCCTAACC-3') were designed to amplify a 285-bp PCR product in the promoter region of exon 1. The Primer 3 (http://frodo.wi.mit.edu/) software was used to make the primer. The amplification of PCR was performed in a 20-µL reaction mixture containing 50 ng templates 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). PCR was carried out under an initial denaturation at 95°C for 5 min, 31 cycles of denaturation at 94°C for 30 s, 52°C annealing temperature for 30 s, extension at 40°C for 30 s, and a final extension at 72°C for 10 min. Later, electrophoresis of the PCR product was performed on 1.5% agarose gels (staining with 200 ng/mL ethidium bromide) using 1X TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2EDTA).

PCR product purification was performed with the Wizard Prep PCR purification kit (Shanghai Bioasia Biotechnology, P.R. China) and sequenced with an ABI PRIZM 3730 DNA sequencer (Perkin-Elmer Shanghai Sangon Biological Engineering Technology, Ltd.). Analyses of the DNA polymorphisms were carried out and the sequence data obtained were compared with the published sequence in the NCBI database (http://www.ncbi.nlm.nih.gov) by the DNAMAN software (version 6.0). The sequence trace of the novel SNP of bovine MyF-5 exon 1 region revealed a T→A mutation at 526 bp using the Chromas software, version 2.33 (http://www.technelysium.com.au/).

Single-strand conformation polymorphism (SSCP) analyses

SSCP was used as a genotyping method in the amplified area of the PCR product. Aliquots of 2 µL PCR products were mixed with 10 µL denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole, and 0.025% bromophenol blue), denaturized at 98°C for 10 min, and then chilled on ice. Thereafter, the denatured DNA was loaded on 8% PAGE in 1X TBE buffer and run under a constant voltage of 120 V for 14 h. The gel was stained with 0.1% silver nitrate, processed and visualized with 2% NaOH (containing 0.1% formaldehyde), according to Zhang et al. (2007).

Statistical analyses

Among the 5 indigenous Chinese *B. taurus* cattle breeds, allele and genotype frequencies of the MyF-5 gene were calculated directly. Hardy-Weinberg equilibrium and differences in allelic and genotypic frequencies were determined by the χ^2 test, using the SPSS software (version 17.0). The population indices, H_E (gene heterozygosity), H_O (gene homozygosity), N_E (effective allele numbers), and PIC (polymorphism information content) of the MyF-5 gene among the five *B. taurus* breeds were calculated by the approaches of Nei and Roychoudhury (1974) and Nei and Li (1979). The association of methods between Myf-5 genotypes and meat quality traits (BFT, WHC, LW, Mb, LEH, RA, LEA, MT) of Chinese *B. taurus* cattle were calculated using the SPSS software (version 17.0) and data are reported as means ± SEM. The following model was applied to analyze the data:

$$Y_{ijkl} = \mu + S_i + Bj + G_k + D_l + b_{ijkl} + e_{ijkl}$$

were Y_{ijkl} is the observed value of meat quality traits; μ is the overall population mean; S_i is the sex effect (i = 1 for male, 0 for female); B_j is the breed effect; G_k is the effect of the k^{th} genotype; D_l is the effect of the first year; b_{ijkl} is the regression coefficient of the slaughter age, and e_{ijkl} is the random residual matching to the observed value.

RESULTS AND DISCUSSION

PCR-SSCP analysis of the MyF-5 gene

A part of exon 1 sequence (285 bp) was amplified by PCR amplification of primer pair MyF5-P1 in all *B. taurus* cattle (Figure 1). PCR-SSCP method allowed the identification of a novel SNP at 526 bp (Gene-Bank accession No. NC_007303). The product exhibited two different patterns. We assigned the AA genotype to those having 3 double-bands, and the AB genotype to those having 3 single-bands (Figure 2). Figure 3 shows a sequencing map of the novel SNP-T>A in the exon 1 promoter region of Chinese *B. taurus* cattle.

Genetic polymorphism of the MyF-5 gene

In the sequencing analysis of A and B alleles prevailed a T→A mutation at 526 bp of the amplified product. The observed mutation, which is identified at exon 1 in the promoter

region of the MyF-5 gene, was the cause of the polymorphism. Therefore, it might play a potential role in affecting the meat quality traits or linkage disequilibrium in the Chinese *B. taurus* cattle.

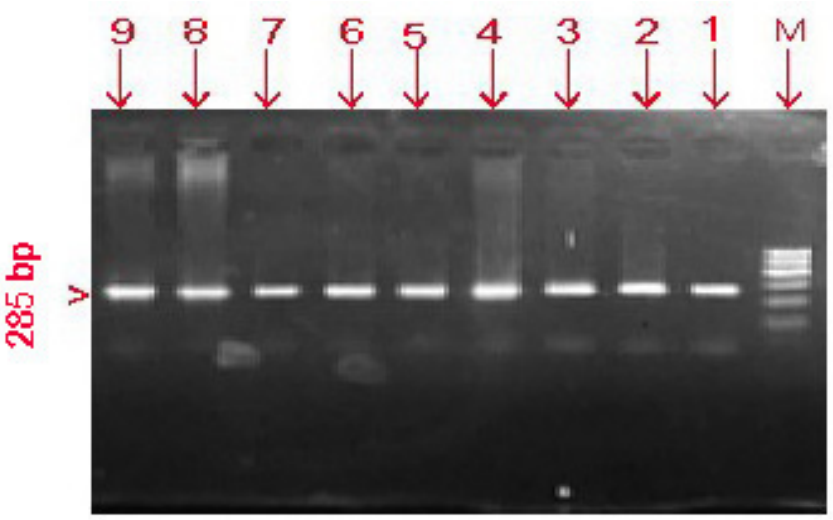

Figure 1. PCR product of 285 bp for exon 1 of the MyF-5 gene and its flanking region. *Lane M* = molecular marker; *lanes 1-9* = PCR products of the MyF-5 gene in the promoter region of exon 1 and its lying region.

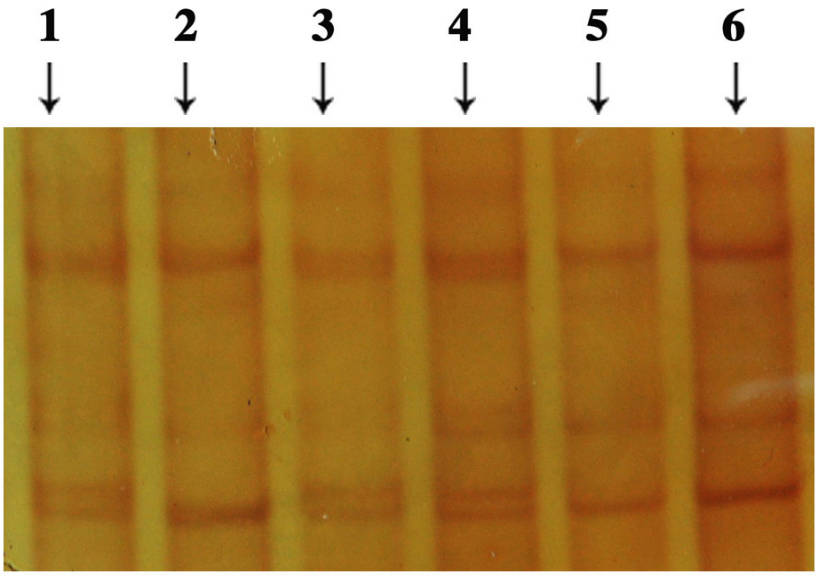

Figure 2. Poly-agarose gel electrophoresis patterns of PCR-SSCP exon 1 of the bovine MyF-5 gene. *Lanes 1*, *3* and *4* = genotype AA; *lanes 2*, *5* and *6* = genotype AB.

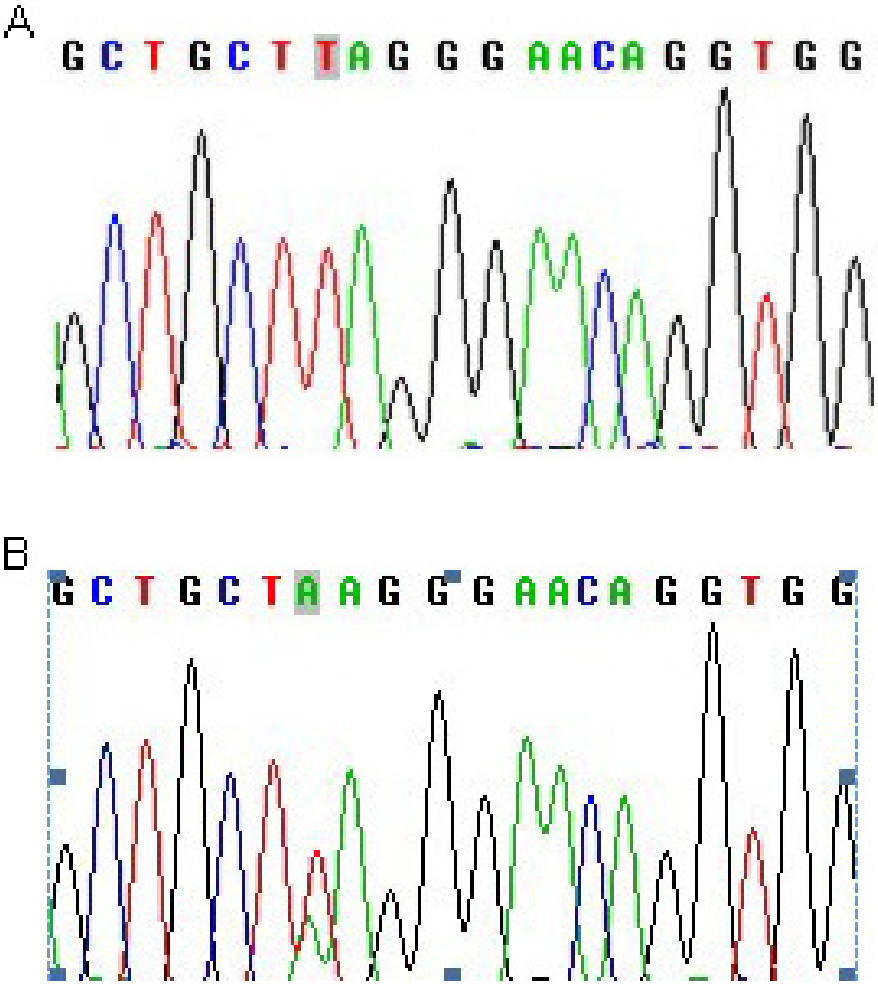

Figure 3. Sequencing map of the novel SNP of the bovine MyF-5 gene exhibiting T>A mutation in the exon-1 region.

The χ^2 test showed that the genotype distributions in 3 breeds (JXR, NY and XN) were in Hardy-Weinberg equilibrium ($P > 0.05$) and these results are similar with those reported by other authors (Zhong et al., 2010; Adoligbe et al., 2011; Wei et al., 2011). However, the populations of LX and QC at the MyF-5 gene locus were not in Hardy-Weinberg equilibrium ($P < 0.01$ and $P < 0.05$, respectively).

The frequency analysis of SNP demonstrated a wide range of allele frequencies in all animal breeds studied (JXR, LX, NY, QC, and XN) from 0.239 to 0.760 (Table 1). We found a very significant difference in the allelic frequency between LX and QC breeds ($P < 0.01$ and $P < 0.05$, respectively). This difference is possible because of the occurrence of random genetic drift due to the low frequency of the B allele. Homozygous null BB in the MyF-5 gene was

not detected in all breeds tested; this may be due to a very low frequency of allele B or maybe homozygous null BB is lethal. However, we did not find any significant difference among the allelic frequencies of JXR, NY and XN (P > 0.05) and this might indicate that the MyF-5 gene locus was under homeostasis followed by the effect of artificial selection, migration and the genetic drift. Allele frequencies of MyF-5-A/B in the five breeds were 0.760/0.239, 0.752/0.247, 0.629/0.370, 0.715/0.284, and 0.750/0.250.

Table 2 shows the genetic diversity of the locus. PIC and H_E values of the NY breed in the loci were higher than that of other population breeds, which suggests that the polymorphism and genetic variation of NY breed were higher than that of other breeds. Furthermore, gene homozygosity varied from 0.534 (NY) to 0.637 (JXR) and N_E ranged from 1.572 (JXR) to 1.873 (NY). The minimum and maximum PIC values were 0.297 (JXR) and 0.357 (NY). As per general trend, PIC is classified into three types: low polymorphism (PIC <0.25), intermediate polymorphism (0.25 < PIC value < 0.5), and high polymorphism (PIC >0.5). Considering the above PIC classification, all *B. taurus* populations showed an intermediate polymorphism level as shown in Table 2. Therefore, our results suggest that the high frequency of the MyF5-A allele could be used to characterize the *B. taurus* breeds.

Table 1. Genotypic and allelic frequencies at the bovine MyF-5 exon 1 and its region.

Breeds	Genotypic frequency (N)		Total	Allelic frequency	
	AA	AB		A	AB
JXR	0.617	0.713	115	0.760	0.239
LX	0.645	0.785	93	0.752	0.247
NY	0.454	0.649	77	0.629	0.370
QC	0.546	0.663	225	0.715	0.284
XN	0.617	0.736	34	0.750	0.250

JXR = JiaXian red; LX = Luxi; NY = Nanyang; QC = Qinchuan, and XN = XiaNan cattle.

Table 2. Population genetic indexes at the MyF-5 gene locus in exon 1 of the Chinese *Bos taurus* breeds.

Breeds	Gene homozygosity	Gene heterozygosity	Effective allele numbers	Polymorphic information content	Hardy-Weinberg equilibrium (χ^2 test)	P values
JXR	0.637	0.363	1.572	0.297	5.140	P > 0.05
LX	0.628	0.372	1.593	0.303	16.5	P < 0.01
NY	0.534	0.466	1.873	0.357	3.957	P > 0.05
QC	0.593	0.407	1.686	0.324	6.519	P < 0.05
XN	0.625	0.375	1.600	0.304	1.817	P > 0.05

JXR = JiaXian red; LX = Luxi; NY = Nanyang; QC = Qinchuan, and XN = XiaNan cattle. HW = $\chi^2_{0.01}$ = 6.635, $\chi^2_{0.05}$ = 3.81.

SNP marker associations

Association studies between gene-specific SNP marker genotypes and meat quality traits were performed using phenotypic data of 440 Chinese *B. taurus* cattle populations. Table 3 shows the comparison of the least square means and the standard errors of meat quality traits. Our results of gene-specific (g.526 T>A) SNP marker suggest a significant association with BFT and MT (P < 0.05). Furthermore, we observed that the genotype AA showed more

values for MT (1.666 ± 0.245) than AB (1.428 ± 0.251). In other words, it is predicted that allele A might be the beneficial allele for meat quality traits. However, no significant effect of this SNP was found in other traits (data not shown).

Table 3. Least square means and standard errors (SE) of the meat quality traits for the MyF-5 gene mutation in Chinese *Bos taurus* cattle.

Meat quality traits	Genotype (means ± SE)		
	AA	AB	P value
Back fat thickness (cm)	11.02 ± 0.115	11.072 ± 0.115	0.043
Meat tenderness	1.666 ± 0.245	1.428 ± 0.251	0.020

Meat tenderness is classified with a score from 1 (extremely tender) to more than 11 (extremely tough). Then, the analyzed beef quality is very tender as suggested by our data.

Currently, breeding goals are shifting from high yield to more meat quality (van Wijk et al., 2005). According to McIlveen and Buchanan (2001), flavor, tenderness and juiciness are considered to be the three most crucial determinants of sensory enjoyment for the United Kingdom consumers. Many reports focused on the association of MyF-5 gene variation with meat quality traits, carcass traits, body measurement traits in pigs and other mammal species. Moreover, some studies have also been performed on body measurement traits or carcass traits and meat quality traits in cattle as previously mentioned [(Korean cattle, Canadian cattle and Chinese (Qinchuan) cattle breed]. However, to our knowledge, no studies on the variation (polymorphism) of the MyF-5 gene and meat quality traits in other Chinese *B. taurus* cattle breeds such as JXR, LX, NY, and XN have been reported. Therefore, the present study was aimed at finding the polymorphism of the MyF-5 gene and its associations with meat quality traits in 5 indigenous Chinese cattle breeds namely JXR, LX, NY, QC, and XN, together called Chinese *B. taurus* cattle. MyF-5 has been considered as a positional candidate gene that inherits QTL impression and has been mapped in BTA5 at 19.0 cM within the QTL region 0-30 cM (Li et al., 2004). Thus, this gene could be considered a possible positional candidate gene for the meat quality traits in Chinese *B. taurus* cattle.

As previously reported in the literature, SNP in the MyF-5 gene has been mentioned to be associated with growth traits in Canadian commercial cattle (Li et al., 2004); growth traits in Chinese (Qinchuan) cattle (Zhang et al., 2007), and carcass traits in Korean (Hanwoo) cattle (Bhuiyan et al., 2009). Moreover, polymorphisms in the porcine MyF-5 gene and its relationship with different meat traits in different pig lines and breeds have also been reported (Venza et al., 2009; Kunhareang et al., 2009; Robakowska-Hyzorek et al., 2010).

The current study is the first report on polymorphism of the MyF-5 gene and meat quality traits in Chinese *B. taurus* populations. Our results suggest new selective information in this regard, i.e., that the exon 1 (g.526 bp) T>A synonymous mutation is significantly associated with back fat thickness and meat tenderness. The association of results between SNP genotypes of the MyF-5 gene and meat quality traits are in agreement with previous research showing that this polymorphism had a significant association with carcass, meat quality, and reproduction traits in different pig lines and breeds (te Pas et al., 1999; Cieslak et al., 2000; Carmo et al., 2005; Wyszynska-Koko et al., 2006; Verner et al., 2007; Humpolícek et al., 2007). The novel SNP (g.526 bp) could result in a synonymous mutation in the MyF-5 gene, which may lead to protein with the same amino acid sequence but different structural and

functional characteristics (Komar, 2007). The degeneracy of the genetic code enables the same amino acid sequences to be encoded and translated in different ways (Kurland, 1991). We know that the genome is highly redundant in terms of tRNA species for each amino acid but it curiously underrepresents a number of specific codons (Shah et al., 2008) in the formation of gene-specific protein.

In conclusion, the present study revealed a novel SNP in the MyF-5 gene exon 1. This SNP (g.526T>A) is significantly associated with back fat thickness and meat tenderness in all 5 Chinese indigenous (*B. taurus*) cattle breeds. Our results confirm the results of the previously reported significant associations and also suggest that this SNP could be used for marker-assisted selection, but a great number of samples would be required for this task.

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