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Association between polymorphism of MyF-5 gene with meat quality traits in indigenous Chinese cattle breeds

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Abstract. Qualitative trait loci for growth and meat quality traits in cattle have been previously mapped in three chromosomal regions of 0 to 30 cM, 55 to 70 cM, and 70 to 80 cM of cattle chromosome 5. Present study was aimed at investigating the new single nucleotide polymorphism (SNP) in MyF-5gene, to evaluate whether this polymorphism affected meat quality traits and to estimate the allelic frequencies from four indigenous Chinese cattle breeds, namely Jia-xian red (JX), Luxi (LX), Nan-yang (NY) and Qinchuan (QC). The result reveals a transversion A→ C at position 1553 was detected in exon2 of MyF-5 gene in cattle, which causes an amino acid substitution (^{1553}glynine/^{1553}proline). Least squares analysis showed that the SNP was significantly associated with live weight (LEH), loin eye height (LEA), loin eye area (LEA) and water holding capacity (WHC) (P<0.05), while no effect of genotype on back fat thickness (BFT), marbling (MB), meat tenderness (MT) and rib area (RA) (P>0.05).Allelic frequencies M/N in four breeds were 0.881/0.118, 0.828/0.171, 0.721/0.279 and 0. 0.784/0.215,respectively. The x^2-test showed that the genotype distributions among three cattle breeds (LX, NY, QC and XN) were not in Hardy–Weinberg equilibrium (P<0.05), while one breed (JX) was in agreement with Hardy–Weinberg equilibrium (P>0.05). Genotype frequencies in two cattle breeds revealed a moderate diversity (0.25<PIC<0.5). Our results suggest that the A1553C SNP of the MyF-5 gene is a possible candidate gene that influences meat quality traits in indigenous Chinese cattle breeds. Moreover, it is also predicted that this SNP could be used in marker assisted selection for meat quality traits in indigenous Chinese cattle breeds.

Keywords: Association; allele frequencies; Chinese cattle; myogenic factor 5; meat quality; mutation.

1. INTRODUCTION

Differences in meat quality are probably due to the variation in different genetical and ecological factors, which interact and determine the course of metabolic reactions in muscle tissue and also in the postmortem conversion of muscle to meat. A candidate gene access may furnish a more channeled understanding of the genetic basis for the manifestation of quantitative divergences between individuals [1]. Myogenesis is a multi-dimensional process involving commitment, proliferation, and specification during embryo growth maturation and function [2] and is chiefly controlled by myogenic determination (MyoD) gene family. MyoD consists of 04 family members (MyF3 (MyoD) 1, MyF-4or Myogenin (MyoG), MyF-5 and MyF-6 genes. Myogenic factor 5 (MyF-5) have been fine mapped for QTLS for birth weight, pre weaning average daily gain, and average daily gain on cattle chromosome 5 [3-4] and three chromosomal regions (0 to 30 cM, 55 to 70 cM, and 70 to 80 cM) were disclosed that are significantly associated with carcass and meat quality traits. The role of MyF-5 and MyF-6 are the considered to be inherent for innovation and growth of straight muscle and to the sustainment of its physical appearance. Hence, they are believed to be candidate genes for growth and meat quality characters [5-6]).Study on candidate genes offers the identification of SNPs in

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genes that most likely cause mutation in a phenotypic trait grounded on physiological and endocrinological[7].Previously, polymorphism in MyF-5 gene were described to be associated with growth traits in Canadian cattle [8]; growth and average daily gain in Korean (Han woo) cattle [9]; growth traits in Chinese (Qinchuan) cattle breed [10]; on growth and carcass traits in Korean (Han woo) cattle [11]. Nevertheless, few researches have been made on the relationship between the polymorphism of MyF-5 gene and meat quality traits. Hence, the objective of this study is to determine the polymorphism of MyF-5 gene, to calculate the allelic, genotypic frequencies and also to determine the polymorphic information index in indigenous Chinese cattle breeds (Chinese Bos tarus).

2. Material and Methods

2.1. Animals and data collection

A total of 516 animals from four different cattle breeds, including JX (n=118), LX (n=128), NY (n=138), and QC (n=132).Animals at the age of 2.5 years were used in this study. The JX animals were obtained from the breeding farm of JX cattle (Jiaxian County, Henan Province, P R China). The LX cattle were acquired from the reserve center of LX (Heze city, Shandong province, P R China). The NY animals were from the breeding center of NY cattle (Nanyang city, Henan Province, P R China). The QC cattle were obtained from the reserve farm of QC (Weinan city, Shaanxi Province, P R China).. The animals were cared for and selected according to the standards of the Canadian Council on Animal care as described by [12].All the animals were used to analyze the allelic frequencies of MyF-5 gene The association analysis of 408 meat quality traits were accounted as previously described [13], aiming on back fat thickness (**BFT**), Live weight (LW), loin eye height (**LEH**), loin eye area (**LEA**), live weight (**LW**), marbling (**Mb**), and meat tenderness (**MT**), rib area (**RA**) and water holding capacity (**WHC**). Mb for meat quality grade was calculated based on a cross-section of the loin muscle between the 12^{th} and 13^{th} rib, which is scored on a scale from 1 to 5.

2.2. DNA extraction and PCR amplification

Five hundred and sixteen blood samples were acquired from four Chinese indigenous cattle breeds. DNA samples were extracted from leukocytes and isolated from acid citrate dextrose (ACD) blood samples (ACD:blood is 1:6), treated with 2% heparin, and stored at -80 C, following standard procedures [14].According to the NCBI database of bovine MyF-5 gene (Gene-Bank accession # NC_007303), one primer pair forward (5' GGGGAGTGGAGAAGCA -3') and reverse (5'- GCAGTTTTGACAGCGTC -3') were designed to amplify a 283-bp PCR product in exon 2 of the MyF-5 gene by using the **program primer 3 (http://frodo.wi.mit.edu/).**

PCR amplifications were performed in a 20-μL reaction mixture containing 50 ng template DNA, 10 pM of each primer, 0.20 mM dNTPs, 2.5 mM $MgCl_2$, and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). PCR was performed under the following conditions: initial denaturation at 95℃for 5 min; 32 cycles of denaturation at 94℃ for 30 s, 58℃ annealing temperature for 30 s, extension at 72℃ for 30 s; and a final extension at 72℃ for 10 min. Electrophoresis of PCR product was performed in 1.5% agarose gels (containing 200 ng/mL ethidium bromide) using 1×TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2EDTA).

The PCR products were purified 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.).DNA polymorphisms were analyzed by comparing the obtained sequence data with the sequences in the NCBI database (http://www.ncbi.nlm.nih.gov) using DNAMAN software (version 6.0). The sequence trace of the novel SNP of bovine MyF-5 exon 2 region revealed A > C mutation at bp 1553 using **Chromas** 2.33 version (http: // www.technelysium.com.au/) software **(Figure 2).**

2.3. PCR-SSCP analyses

For single-strand conformation polymorphisms (SSCP), Aliquots of 4 μL of the above PCR products were combined with 8 μL of denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), incubated at 98℃ for 10 min, and then chilled on ice. Denatured DNA was

loaded onto a 10% PAGE gel in 1×TBE buffer and run under a constant voltage of 120V for 12 h. Finally, the gel was stained with 0.1% silver nitrate, and developed and visualized with 2% NaOH solution (containing 0.1% formaldehyde), according to [10].

2.4. Statistical analyses

Allele frequencies and genotype frequencies of the 4 indigenous Chinese Bos tarus cattle breeds were calculated directly. Hardy Weinberg equilibrium and differences in allelic and genotypic frequencies were calculated by $\chi 2$ test in SPSS software (version 17.0). Population indices likewise, He (gene heterozygosity), Ho (gene homozygosity), Ne (effective allele numbers) and PIC (polymorphism information content) of the MyF-5 gene in 04 Bos taurus breeds were estimated by utilizing the approaches of [15-16].Association analyses between MyF-5 genotypes and meat quality traits (BFT,WHC,BT,LW, LEH, RA, LEA ,MT) of Chinese Bos tarus cattle were calculated using SPSS software (version 17.0) and the data are shown as ± S.E.M. Following model was applied to analyze the data:

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

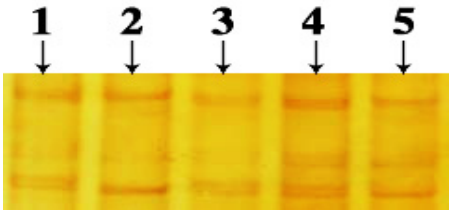
Here: Y_{ijkl} represents the observed value of meat quality traits, μ for the overall population mean, S_i s for the sex effect (i = 1 for male, 0 for female), B_j stands for the breed effect, G_k stands for the effect of the k_{th} genotype, D_l denotes the effect of the first year, and b_{ijkl} is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

3. Results

3.1. SNP and genotype frequencies of different populations

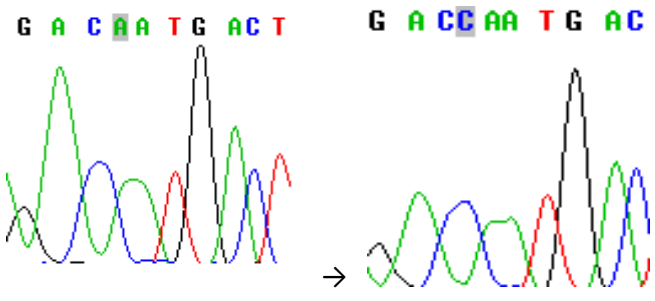
A 283-bp of amplified product prevailed in all animals probed**.** PCR-SSCP amplification and sequencing of the 283-bp fragment, we discovered a single novel SNP at A1553C in exon2 of MyF-5 gene (Gene-Bank accession number NC_007313.3) in 561animals. PCR-SSCP results are shown in **Figure 1**. This A→C mutation at bp 1553 was a point mutation (transversion), which induced a change from serine ^{1553}glynine/^{1553}proline. The SNP (A1553C) in all the breeds that we analyzed in this study exhibited two different patterns **(Figures 2**). For identification, we assigned them MM with two single bands and one double band and MN with three double bands. Sequencing map of the novel SNP of bovine MyF-5 exon 2 region also showed a synonymous mutation A>C **(Figure 2)**.

Figure 1 The poly agarose gel electrophoresis patterns of PCR-SSCP exon2 of the bovine MyF-5 gene



Here 1, 3&4 represent the MN genotype, while 2 & 5 represent MM genotype

Figure 2 The sequencing map of the novel SNP of bovine MyF-5 gene in exon2 region. This map reveals' an A→ C mutation at 1553-bp



Furthermore, allelic frequencies of the SNP were calculated by the x2 test in all breeds studied (JX, LX, NY and QC) and results varied from 0.721to 0.881 (Table 1).The x2 test revealed that the genotype distributions among 3 cattle breeds, LX, NY, QC and XN, did not agree with Hardy–Weinberg equilibrium and showed significant differences in allelic frequencies (P<0.05). However, JX were in Hardy–Weinberg equilibrium and did not show any significant difference in allelic frequency (P>0.05), as shown in Table 1. The genotypic frequencies among 03 cattle breeds showed small diversity (0.25<PIC<0.5). AA genotype was the dominant genotype in all populations examined, ranging from 0.442 to 0.762, and the MN genotype frequency was lower in all breeds studied, ranging from 0.237to 0.558.

Table 1 Allele, genotype frequencies and population genetic indices at the bovine MyF-5 gene locus in four Chinese indigenous cattle breeds

Breeds	Genotype frequency (N)		Total	Allele frequency		Effective allele number	PIC	He	X^2 (HWE)	P(X)
	MM	MN		M	N	Ne				Value
JX	0.762(90)	0.237(28)	118	0.881	0.118	1.264	0.187	0.209	1.148	P>0.05
LX	0.656(84)	0.343(44)	128	0.828	0.171	1.398	0.244	0.284	4.339	P<0.05
NY	0.442(61)	0.558(77)	138	0.721	0.279	1.673	0.321	0.402	20.661	P<0.01
QC	0.568(75)	0.431(57)	132	0.784	0.215	1.511	0.281	0.338	10.008	P<0.01

JX = Jia-xian red cattle; LX = Luxi cattle; NY=Nan yang cattle and QC = Qinchuan cattle.

HW = Hardy-Weinberg equilibrium; $\chi^2_{0.01}$=6.635, $\chi^2_{0.05}$=3.81

3.2. SNP marker associations

Association studies between gene specific SNP marker genotypes and meat quality traits were performed using phenotypic data of 408 Chinese Bos tarus cattle. Our results of gene specific (g.1553 A>C) SNP marker suggests a significant associations with live weight (LEH), loin eye height (LEA), loin eye area (LEA) and water holding capacity (WHC) (P<0.05), while no effect of genotype on back fat thickness (BFT), marbling (MB), meat tenderness (MT) and rib area (RA) (P>0.05). The results of association analysis are showed in **Table 2.**

Table 2 Least square means and standard errors of the carcass and meat quality traits found for the genotypes of the MyF-5 gene polymorphism in Chinese indigenous cattle.

Genotype	Meat quality traits (cm, mean ± SE)			
	LW	LEH	LEA	WHC
MM	19.4±8.60+	2.6±0.226	27.9±0.023	0.027±0.46
MN	18.3±6.56	1.0±0.493	18.8±0.975	0.021±0.01
P value	0.035	0.042	0.024	0.049

Here; LW = live weight; LEH =loin eye height: LEA = loin eye area; and WHC = water holding capacity.

4. Discussion

Currently, breeding goals are shifting from high yield to more meat quality traits [17].A number of studies focused on association of MyF-5 gene variation with meat quality traits, carcass traits, body measurement traits in pigs and other mammal's species. Some research have also been done on Body measurement traits or carcass traits and meat quality traits in cattle as reported previously (Korean cattle, Canadian cattle and Chinese (Qinchuan) cattle breed. To our best knowledge, however, no studies on the variation (polymorphism) of MyF-5 gene and meat quality traits in other Chinese Bos tarus cattle breeds

such as Jiaxianred, Luxi, Nan yang and Xia Nan. So, the present study was aimed at finding the polymorphism of MyF-5 gene and its associations with meat quality traits in 4 indigenous Chinese cattle breeds namely Jiaxianred, Luxi, Nanyang and Qinchuan together called Chinese Bos tarus cattle.MyF-5 has been considered as a positional candidate gene, which inherits QTL impression and has been mapped in BTA5 at 19.0 cM within the QTL region 0–30 cM [8].Moreover, this gene is a possible positional candidate gene for the meat quality traits in Chinese Bos tarus cattle.

Previously, SNP in MyF-5 gene have been mentioned to be associated with growth traits in Canadian commercial cattle's [8]; growth traits in Chinese (Qinchuan) cattle breed [10]; carcass traits in Korean (Hanwoo) cattle [11].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 [18-19].

The Present study is the first report on polymorphism of MyF-5 gene and meat quality traits in Chinese Bos tarus populations. Our results suggested a new selective information in this respect that the exon2-g.1553 bp A>C synonymous mutation is significantly associated with live weight , loin eye height , loin eye area and water holding capacity, while showed some non-significant associations with back fat thickness, marbling , meat tenderness and rib area($P>0.05$) The association results between this SNP genotypes of MyF-5 gene and meat quality traits are in agreement with previous probes that this polymorphism had significant association with carcass, meat quality, and reproduction traits in different pig lines and breeds have been identified [2,6,20,21,22,23,].Our novel SNP (g.1553- bp) could result in a synonymous mutation in MyF-5 gene, which maybe leads to protein with the same amino acid sequence but different structural and functional characteristics [24].Genetic code degeneracy enables the same amino acid sequences to be encoded and translated in different ways [25].It is known that the genome is highly redundant in terms of tRNA species for each amino acids but mysteriously under represents a number of specific codons [26] so in the formation of gene specific protein.

In conclusion, the present study revealed a novel SNP in MyF-5 gene exon 2. This SNP g.A1453>C is significantly associated with live weight, loin eye height, loin eye area and water holding capacity, in all 4 Chinese indigenous (Bos tarus) cattle breeds ,while showed non-significant associations with back fat thickness, marbling , meat tenderness and rib area. Our results confirm legion of the previously reported significant associations. It is also suggested that this SNP could be used for marker-assisted selection but a huge number of samples would be required for this job.

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