The Effect of Myogenic Factor 5 Polymorphism on the Meat Quality in Chinese Bos Taurus

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Summary

In the present study, we evaluated polymorphism of myogenic factor 5, involved in growth and meat quality traits. Based on PCR-SSCP technology, a novel missense substitution SNP (single-nucleotide polymorphism) g.1142 A > G was identified in the intron1 region of the MyF-5 gene, it causes an amino acid substitution (^{1142}Glutamine/ Glycine^{1142}). Allele frequencies, gene heterozygosity, effective allele number and polymorphism information content of the bovine MyF-5 SNP in three population breeds were determined and evaluated by the $\chi 2$ test. Results showed that the polymorphism distribution was not similar in all of the three Bos taurus breeds, the genotype distributions of two cattle breeds Jia xian red and Nanyang did not agree with Hardy–Weinberg equilibrium ($P < 0.01$); one breed Qinchuan did not deviate significantly from Hardy–Weinberg equilibrium ($P > 0.05$). The A/G allelic frequencies in these breeds were 0.797/0.202, 0.770/0.229, 0.863/0.136 respectively. The genotype frequencies in Jia xian red and Nanyang cattle breeds showed moderate diversity (0.25< polymorphism information content <0.5). Furthermore, least squares analysis revealed significant effects of genotype on intramuscular fat, rib area and water holding capacity in 510 individuals ($P < 0.05$). Our result suggests that A1142G SNP can be used as an efficacious genetic marker for meat quality traits in native Chinese cattle breeds (Bos taurus) but a much large number of animals are required for Marker assisted selection.

Key words

allelic frequencies, Chinese Bos taurus, myogenic factor 5, meat quality, single nucleotide polymorphism

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Aim

Variation in meat quality is probably due to multiple factors that affect the quality of meat, including the way animals are fed, managed, slaughtered and both carcass handling and processing post-slaughter. Although, there is often stress on the management systems that can be employed to meet market specifications there has, until recent years, been little emphasis on factoring molecular or biological components of meat quality. Here, we studied the genetic basis of meat science research. Myogenesis is related to the numbers of myofibers in muscle. It is completely embryonic process and it is regulated by MyoD gene family (Olson, 1990). The MyoD gene family consists of four structurally and functionally related genes: MyoD1 (MyF-3), MyoG (Myogenin), MyF-5 and MyF-6 (herculin). Moreover, all the genes consist of three exons and share homology within the region coding for basic helix-loop-helix (bHLH) domain (Sehara et al., 1990). MyF-5 gene is considered as a candidate gene for the meat production and quality traits because of its probable role in muscle fiber development (Wyszynska-Koko et al., 2006; Verner et al., 2007). Study on the candidate genes underlying within a QTL region are positional candidate genes and association of these genes with quality traits provides an excellent opportunity for marker assisted selection (Khatib et al., 2007; Dario et al., 2009). MyF-5 gene has been mapped in a QTL position between 0 and 30 cM on BTA5 (Li et al., 2002). Therefore, this gene is a strong candidate gene for meat quality traits in Chinese Bos taurus. In cattle, MyF-5 gene has a length of 3236 bp, with 3 exons and 2 introns (Gene Bank accession. No. NC_007303). In previous research, polymorphism in MyF-5 gene was described to be associated with: growth traits in Canadian cattle (Li et al., 2004); growth and average daily gain in Korean (Han woo) cattle (Chung and Kim, 2005); growth traits in Chinese (Qinchuan) cattle breed (Zhang et al., 2007); and growth and carcass traits in Korean (Han woo) cattle (Bhuiyuan et al., 2009). Although, few studies have been done on the relationship between the polymorphism of MyF-5 gene and meat quality traits, hence, the objective of current research is to determine the polymorphism of MyF-5 gene, to evaluate the allelic, genotypic frequencies and also to determine the polymorphic information index in Chinese indigenous cattle (Chinese Bos taurus cattle).

Material and methods

Animals and data collection: a total of 672 animals from three different cattle breeds, including Jiaxian red, JX (n=230), Nanyang, NY (n=277) and Qinchuan, QC (n=165) each 2 years of age were used to analyze the allelic frequencies of MyF-5 gene. The JX animals were obtained from the breeding farm of JX cattle (Jiaxian county, Henan 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 meat quality traits of 510 samples were calculated according to the criterion GB/T 17238-1998 cutting standard of fresh and chilled beef in China (China standard publishing house). The traits included were: back fat thickness (BFT), intramuscular fat (IF), rib area (RA), meat tenderness (MT), marbling (Mb) and water holding capacity (WHC). Mb for meat quality grade was calculated based on a cross-section of the loin muscle between the 12th and 13th rib, which is scored on a scale from 1 to 5.

DNA extraction and PCR amplification

Three indigenous Chinese cattle breeds were used for 672 sampling and DNA was extracted from leucocytes and assorted from acid citrate dextrose (ACD) blood samples (ACD- blood is 1:6), then treated with 2% heparin, and ultimately stored at -80°C, observing the standard method prescribed by Sambrook and Russell (2002). According to the bovine MyF-5 gene (Gen-Bank accession No, NC_007303), one pair of PCR primers (forward: 5' GGCCTCCACTGTCCCA 3' and 5' GCAGTGCTTGCCCACC 3') was designed to amplify a 175- bp PCR product in intron1. Polymerase chain reaction (PCR) amplifications were performed in 20 µL reaction mixture containing 50 ng DNA template, 10 pM of each primer, 0.20 mM dNTP, 2.5 mM MgCl2 and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). The cycling protocol was 5 min at 95°C, 32 cycles of 94°C for 30 s, 56°C annealing for 30 s, 72°C for 30 s, with a final extension at 72°C for 10 min. PCR products were electrophoresed on 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).

Single strand conformation polymorphism (SSCP) and sequencing

PCR products were analyzed for single-strand conformation polymorphisms (SSCP), aliquots of 2 µL of above PCR products were mixed with 8 µL of the denaturing solution (95% formamide, 25mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), incubated at 98°C for 10 min and then chilled on ice. Denatured DNA was loaded on 10% PAGE gel in 1×TBE buffer and constant voltage of 121V for 14 h. The gel was stained with 0.1% silver nitrate and visualized with 2% NaOH solution (containing 0.1% formaldehyde) according to Zhang et al. (2007). To confirm the results based on PCR-SSCP technique, the PCR products from the mix DNA template were sequenced in both directions. 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).

Statistical analyses

Allele frequencies, genotype frequencies, Hardy–Weinberg equilibriums, gene homozygosity (Ho), gene heterozygosity (He), effective allele numbers (Ne) and polymorphism information content (PIC) were statistically analyzed according to the procedure of Nei and Roychoudhury (1974) and Nei and Li (1979). The relationship between SNP marker genotypes of MyF-5 gene and meat quality traits; back fat thickness, intramuscular fat, rib area, meat tenderness, marbling and water holding capacity was performed by the software SPSS (version 17.0) as per the following statistical model:

$$Yijk = \mu + G_j + A_i + S_k + E_{ijk},$$

where: Y_{ijk} is the observation for the meat quality traits, μ is the overall mean for each trait, G_j is the genotype effect, A_i is the fixed effect of age, S_k is the fixed effect of sex, E_{ijk} is the random error.

Results and discussion

PCR-SSCP analysis of the MyF-5

Based on PCR-SSCP method, PCR product of 175-bp for the intron1 of the MyF-5 gene has been amplified in all the experimental animals (Figure 2).The product exhibited two different patterns. We named the pattern with single bands AA and the other with two bands AG (Figure1). The sequencing trace of the novel SNP of bovine MyF5 region revealed an A>G mutation at 1142-bp (Figure 3).

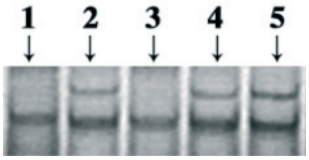

Figure 1. The poly agarose gel electrophoresis patterns of PCR-SSCP intron1 of the bovine MyF-5 gene (1 and 3 represent AA genotype while the 2, 4 and 5 represent AG genotype)

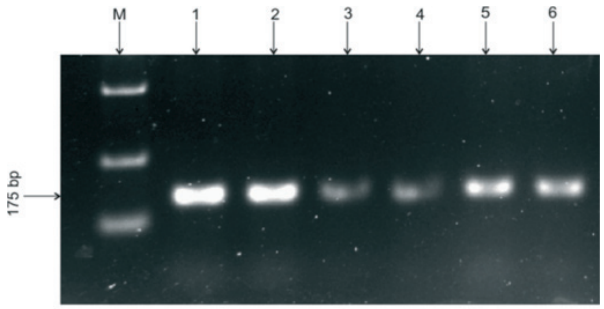

Figure 2. PCR product of 175-bp for the intron1 of the MyF-5 gene and its flanking region

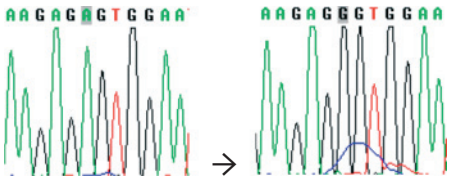

Figure 3. Sequencing map of the novel SNP of the bovine MyF-5 intron 1 region (This map reveals A→G mutation at 1142-bp.)

Genetic polymorphism of bovine MyF_5 gene

Sequence analysis of G and A allele of this study revealed a A >G synonymous mutation at 1142-bp position of the amplified product. The genetic diversity of the locus was then calculated (Table 1). The results suggested that the mutant allele G is present in three breeds and had lower frequency compared with the wild allele A in these populations. The A/G allelic frequencies were 0.797/0.202, 0.770/0.229, 0.863/0.136 respectively. He, Ne and PIC of NY breed in the locus were higher than in the other populations, which implied that the polymorphism and genetic variation of NY breed were higher than that of others breeds. $\chi 2$ test showed that the polymorphism distribution was not similar in all of the three Bos taurus breeds, the genotype distributions of two cattle breeds Jia xian red and Nanyang did not agree with Hardy–Weinberg equilibrium ($P < 0.01$); one breed Qinchuan did not deviate significantly from Hardy–Weinberg equilibrium ($P > 0.05$). The genotype frequencies in Jia xian red and Nanyang cattle breeds showed moderate diversity (0.25< polymorphism information content <0.5). The results are shown in Table 1.

Association of the genotypes on meat quality traits

Six meat quality traits were analyzed by comparison between genotypes of 510 individuals and their phenotypic data. The results of association analysis of the gene-specific marker are shown in Table 2. There were significant effects on Intramuscular fat, rib area and water holding capacity ($P < 0.05$). Moreover, it was also observed that the genotype AA showed more values for intramuscular fat, rib area and water holding capacity than AG respectively. 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 on back fat thickness, marbling and meat tenderness traits (data are not shown).

Currently, breeding goals are shifting from high yield to more meat quality concerned attributes (Van Wijk et al., 2005). According to McIlveen and Buchanan (2001) flavor, tenderness and juiciness are considered to be the three most crucial determinatives of sensory enjoyment for the United Kingdom consumers. Some 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. Furthermore, some research have also been done on body measurement traits or carcass traits and meat quality traits in cattle as mentioned previously (Korean cattle, Canadian cattle and Chinese (Qinchuan) cattle breed. To our best knowledge, yet, no other research have been done on the mutation (polymorphism) of MyF-5 gene and meat quality traits in other Chinese Bos taurus

Table 1. Genotypic and allelic frequencies at the MyF-5 gene intron 1 region in different bovine populations

Breeds	Genotype frequency (N)		Total	Allele frequency		Effective allele number, Ne	PIC	He	X2 (HWE)	P(X) Value
	AA	AG		A	G					
JX	0.595(137)	0.404(93)	230	0.797	0.202	1.476	0.270	0.322	14.769	P<0.01
NY	0.541(150)	0.458(127)	277	0.770	0.229	1.546	0.290	0.353	24.503	P<0.01
QC	0.727(120)	0.272(45)	165	0.863	0.136	1.308	0.207	0.235	3.020	P>0.05

HW = Hardy-Weinberg equilibrium; $\chi 20.01=6.635$, $\chi 20.05=3.841$. The P values for JX and NY was less than 0.01 and for QC were greater than 0.05. PIC = Polymorphism information content; JXR = Jia-xian red cattle; NY=Nan yang cattle; QC = Qinchuan cattle

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)		
	IF	RA	WHC
AA	1.321±0.14	1.660±0.226	1.21±0.017
AG	1.020±0.013	1.560±0.493	1.02±0.007
P value	0.01	0.042	0.024

IF = intramuscular fat; RA = rib area;
LEA and WHC = water holding capacity

cattle breeds such as Jiaxianred and Nan yang. So, the current study was proposed at finding the polymorphism of MyF-5 gene and its associations with meat quality traits in three indigenous Chinese cattle breeds namely Jiaxianred, Nanyang and Qinchuan, together called Chinese Bos taurus cattle. MyF-5 has been recognized 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 (Li et al., 2004). Afterwards, this gene is a possible positional candidate gene for the meat quality traits in Chinese Bos taurus cattle.

In earlier research, SNP in MyF-5 gene have been cited to be associated with growth traits in Canadian commercial cattle's (Li et al., 2004); growth traits in Chinese (Qinchuan) cattle breed (Zhang et al., 2007); carcass traits in Korean (Hanwoo) cattle (Bhuiyan et al., 2009). Furthermore, 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 present research is the first report on polymorphism of MyF-5 gene and meat quality traits in Chinese Bos taurus populations. Our results suggested a new selective information in this regard that the intron1- g.1142 bp A>G synonymous mutation is significantly associated with Intramuscular fat, rib area and water holding capacity. Association outcomes between this SNP genotypes of MyF-5 gene and meat quality traits are in agreement with previous investigations that have been identified significant association of this polymorphism 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; Humpolíček et al., 2007).

Conclusions

In conclusion, the present study revealed a novel SNP in MyF-5 gene intron1. This SNP g.1142 bp A>G is significantly associated with Intramuscular fat, rib area and water holding capacity in all three Chinese indigenous (Bos taurus) cattle breeds. Our research confirms legion of the previously reported significant associations. It is also suggested that this SNP could be used for marker-assisted selection but a lot of samples would be required for this research work.

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