

Association between *IGF-IR*, *m-calpain* and *UCP-3* gene polymorphisms and growth traits in Nanyang cattle

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Abstract The objective of this study was to estimate the allele and genotype frequencies of the *IGF-IR/TaqI*, *m-calpain/HhaI*, and *UCP-3/BglII* polymorphisms and to determine associations between these polymorphisms and growth traits in Chinese indigenous cattle breeds. Genotyping was performed on 321 animals including 135 Nanyang, 80 Qinchuan, and 106 Jiaxian cattle. No significant differences in growth traits were observed between the genotypes of *IGF-IR/TaqI* polymorphism in Nanyang cattle. The *m-calpain/HhaI*, and *UCP-3/BglII* polymorphisms were associated with body weight, withers height, and body length of 6 months ($P < 0.05$ or $P < 0.01$), body length of 18 months ($P < 0.05$), and body length and heart girth of 24 months ($P < 0.01$) in Nanyang cattle. In addition, Nanyang cattle aged 12 months with AB genotype of *m-calpain/HhaI* polymorphism had higher body length and heart girth than those with BB genotype ($P < 0.01$). The withers height were greater ($P < 0.01$) in Nanyang cattle aged 12 months with genotype AB of *UCP-3/BglII* polymorphism than those with genotype BB. The Nanyang cattle aged 24 months with genotype AA had higher withers height than those with genotype BB ($P < 0.05$). In

conclusion, the *m-calpain/HhaI* and *UCP-3/BglII* polymorphisms may be used as DNA markers for selection in the breeding process of Nanyang cattle.

Keywords Beef cattle · Growth traits · Association analysis

Introduction

The main objective of the application of candidate gene strategy to animal genetic improvement currently is to identify and analyze polymorphisms of genes involved in the main metabolic pathways related to animal growth and distribution of nutrients to different tissues. Such investigations have permitted the verification of associations between genotypes and production traits in cattle [1–3]. Insulin-like growth factor type I receptor (*IGF-IR*) is the main receptor of IGFs, mediating the transduction of metabolic signal of cell proliferation, bone growth and protein synthesis in the growth hormone-insulin-like growth factors pathways. Therefore, *IGF-IR* plays a key role in the regulation of the metabolism and physiology of mammalian growth [4]. As a member of calpain family, *m-calpain* may be involved in cytoskeletal and extracellular matrix reorganization in myoblasts, and also functioned in limited proteolysis of proteins like desmin, filamin, talin, vimentin, fibronectin etc., such events being absolutely necessary for myoblast fusion [5, 6]. Uncoupling protein (*UCP*) gene family involved in energy metabolism of body, and had significant effects on energy balance related traits, such as body weight resting metabolic rate, and efficiency of food conversion etc. [7]. Liu et al. [8] found *UCP-3* gene polymorphisms may contribute to body mass index (BMI) in the Caucasian population. Rudofsky et al. [9] found that

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human UCP-3 gene involved in energy balance and is associated with obesity and diabetes.

Nanyang, Qinchuan, and Jiaxian cattle are three of the eight best cattle breeds for beef production in China. Due to their uniqueness, these breeds have been designated as nationally protected genetic resources. Nanyang, Qinchuan, and Jiaxian cattle have been the representatives of Chinese cattle breeds for their good performance traits and especially fleshy characteristics. For thousands of years, Nanyang, Qinchuan, and Jiaxian breeds, famous for their large body size and excellent farming ability, have been the main labor forces in agriculture and important beef sources. However, with the development of science and technology and the ever-increasing mechanization of farming, these good farming abilities have become obsolete. Compared with imported commercial beef cattle breeds, these three breeds have several drawbacks, such as an underdeveloped hind hip, slower growth rate and poor milk production. In an effort to adapt to the development of a commercial economy, it has become necessary for these three breeds to improve meat performance while maintaining their other excellent inherent characteristics. The objectives of the present study were to estimate the allele and genotype frequencies of *IGF-IR*, *m-calpain* and *UCP-3* gene polymorphisms of three breeds and to determine associations between these polymorphisms and cattle growth traits.

Materials and methods

Sampling and DNA extraction

A total of 321 female cattle, representing Nanyang (135), Qinchuan (80), Jiaxian (106), were included in the study. Nanyang cows were fed on an ad libitum concentrate and straw on 24 months of age, after weaning at 6 months age. Nanyang animals were all the same age and growth traits were measured every 6 months from 6 to 24 months according to methods of Chen [10]. Blood samples were collected from the three cattle breeds, and genomic DNA was isolated from blood samples using standard method.

PCR–RFLP and genotyping

Three cattle breeds were genotyped for the *IGF-IR*, *m-calpain* and *UCP-3* genes by PCR–RFLP. Table S1 shows the gene loci studied, the primers and annealing temperatures used for the amplification of the regions of interest. For determination of the A and B alleles of the *IGF-IR* gene, a 625 bp segment was amplified, which showed a polymorphism at the *TaqI* restriction site [11] located in an intron. According to Zhang et al. [12] and Juszczuk-Kubiak et al. [13], to determine the A and B

alleles of the *m-calpain* gene, a 1800 bp fragment including the sequences from exon 4 through introns up to exon 8 was amplified and digested with *HhaI*. The A and B alleles of *UCP-3* gene were identified based on the amplification of a 481 bp fragment corresponding to the exon 3 portion, followed by digestion with the restriction enzyme *BglII* based on Li [14].

Statistical analysis

Gene frequencies were determined for each breed by direct counting. Statistical analysis of associations between genotypes and growth traits of Nanyang heifers was performed, using SPSS (version 16.0). The model applied was:

$$Y_{ij} = \mu + A_i + G_j + e_{ij}$$

where Y_{ij} was the trait measured on each of the ij th animal, μ was the overall population mean, A_i was fixed effect due to the i th age, G_j was the fixed effect associated with j th genotype and e_{ij} was the random error (animal as a random effect with pedigree relationships between animals).

Results

Genotype and allele frequencies

Two genetic variants (A and B) of the *IGF-IR/TaqI* polymorphism were observed in the three breeds studied. Genotype AA was characterized by the presence of two restriction fragments of 580 and 45 bp, while genotype BB was determined by the presence of three restriction fragments of 410, 170, and 45 bp. AB individuals presented four fragments of 580, 410, 170, and 45 bp (Fig. 1). Two different alleles (A and B) were found for the *m-calpain/HhaI* polymorphism in the three breeds. Genotype AA was characterized by the presence of three restriction fragments of 900, 620, and 280 bp. Genotype BB was determined by the presence of two restriction fragments of 1520 and 280 bp. Heterozygous individuals (AB) presented four fragments of 1520, 900, 620, and 280 bp (Fig. 2). Two alleles, A and B, were detected for the *UCP-3/BglII* polymorphism in the three breeds. Genotype AA was characterized by the presence of one fragment of 481 bp, while genotype BB showed fragments of 260 and 221 bp. Heterozygous individuals (AB) were characterized by the presence of three fragments of 481, 260, 221 bp, corresponding to a combination of the two homozygous patterns (Fig. 3).

The allele and genotype frequencies of the *IGF-IR/TaqI*, *m-calpain/HhaI* and *UCP-3/BglII* polymorphism obtained for the three breeds are shown in Table 1. Segregation of the *IGF-IR/TaqI*, *m-calpain/HhaI* and *UCP-3/BglII*

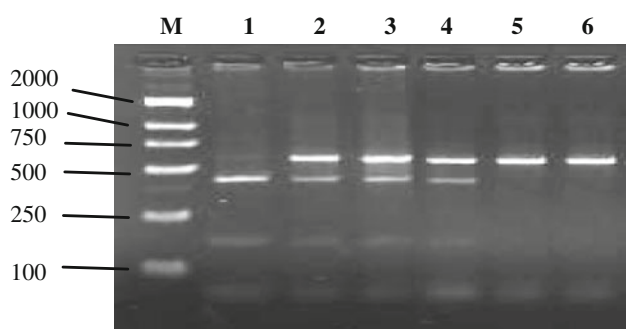


Fig. 1 *TaqI*-RFLP patterns of *IGF-IR* locus Lane 5 and 6, AA genotype; 1, BB genotype; 2, 3 and 4, AB genotype; M, DL2000 Marker

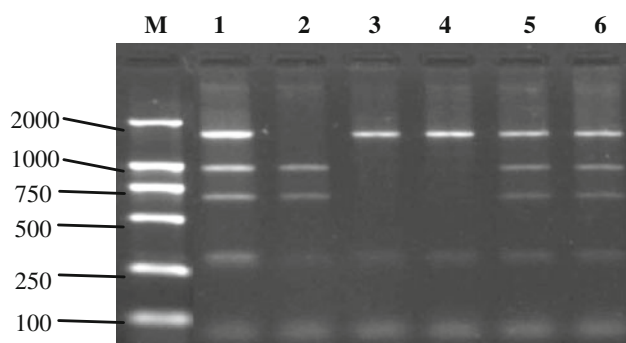


Fig. 2 *HhaI*-RFLP patterns of *m-calpain* locus Lane 2, AA genotype; 3 and 4, BB genotype; 1, 5 and 6, AB genotype; M, DL2000 Marker

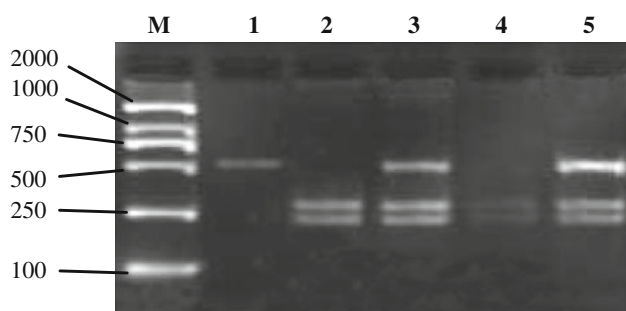


Fig. 3 *BglII*-RFLP patterns of *UCP-3* locus Lane 1, AA genotype; 2 and 4, BB genotype; 3 and 5, AB genotype; M, DL2000 Marker

Table 1 Allele and genotype frequencies of three gene loci polymorphisms in three bovine breeds

Breeds	Gene loci	Genotypes frequencies			Allele frequencies	
		AA	AB	BB	Alle A	Allel B
Nanyang	<i>IGF-IR/TaqI</i>	0.6889	0.2963	0.0148	0.8370	0.1630
	<i>m-calpain/HhaI</i>	0.1111	0.4815	0.4074	0.3519	0.6481
	<i>UCP-3/BglII</i>	0.2370	0.6296	0.1333	0.5519	0.4481
Qinchuan	<i>IGF-IR/TaqI</i>	0.7313	0.1791	0.0896	0.8209	0.1791
	<i>m-calpain/HhaI</i>	0.0750	0.4500	0.4750	0.3000	0.7000
	<i>UCP-3/BglII</i>	0.0656	0.6557	0.2787	0.3934	0.6066
Jiaxian	<i>IGF-IR/TaqI</i>	0.6863	0.2843	0.0294	0.8284	0.1716
	<i>m-calpain/HhaI</i>	0.0472	0.3396	0.6132	0.2170	0.7830
	<i>UCP-3/BglII</i>	0.2268	0.6804	0.0928	0.5670	0.4330

polymorphism was observed and all three genotypes of each polymorphism were presented in the three breed studied. In *IGF-IR/TaqI* polymorphism, the allele A showed a significantly higher frequency than that of the B allele and genotype BB showed a low frequencies in all three breeds. In *m-calpain/HhaI* polymorphism, the frequency of allele B was significantly higher than those of allele A and the frequency the AA genotype was low in all three breeds. In *UCP-3/BglII* polymorphism, the frequency of AB genotype were higher in the studied breeds. In Nanyang and Jiaxian breeds, the frequency of AA genotype were higher than those of BB genotype, while in Qinchuan breed the frequency of AA genotype was lower than that of BB genotype.

Association analysis

To investigate the effects of the polymorphisms, we analyzed the relationship of *IGF-IR/TaqI*, *m-calpain/HhaI* and *UCP-3/BglII* genotypes for the effects on variation in growth traits in 135 Nanyang cows from a breeding farm. Among the three polymorphisms, the *m-calpain/HhaI* and *UCP-3/BglII* polymorphisms were significantly associated with growth traits (Table 2 and 3). There was no association detected between growth traits and *IGF-IR/TaqI* polymorphism (Table S2). Only genotypes AA and AB were considered in the association analysis between *IGF-IR/TaqI* polymorphism genotypes and growth traits due to the shortage of BB homozygote animals. The *m-calpain/HhaI* polymorphism was significantly associated in all four traits studied at 6 months, body length and heart girth at 12 months, body length at 18 months, and body length and heart girth at 24 months, with the heterozygous AB genotype being more favorable than homozygous AA or BB genotype ($P < 0.05$ or $P < 0.01$). Significant associations between the *UCP-3/BglII* polymorphism and growth traits at different growth stages were observed (Table 3). The body weight, withers height and body length were greater

Table 2 The LSM and SE between genotypes of the m-calpain/*HhaI* polymorphism in Nanyang cattle

Growth stage	Genotype	Body weight (kg)	Withers height (cm)	Body length (cm)	Heart girth (cm)
6 months	AA	152.923 ^a ± 5.040	104.308 ^a ± 1.399	103.615 ^a ± 1.513	127.923 ^{ab} ± 1.961
	AB	165.531 ^b ± 2.596	107.265 ^b ± 0.721	107.327 ^{bb} ± 0.780	130.898 ^b ± 1.010
	BB	153.216 ^a ± 2.988	104.622 ^a ± 0.829	103.351 ^{aA} ± 0.897	125.946 ^a ± 1.162
12 months	AA	215.385 ± 6.224	113.769 ± 1.068	115.615 ^{AB} ± 1.955	139.154 ^{AB} ± 2.059
	AB	227.204 ± 3.206	114.571 ± 0.550	119.122 ^B ± 1.007	143.367 ^B ± 1.061
	BB	219.000 ± 3.740	113.222 ± 0.642	114.056 ^A ± 1.175	138.750 ^A ± 1.238
18 months	AA	299.692 ± 8.555	122.154 ± 1.821	129.692 ^{ab} ± 1.846	154.462 ± 2.326
	AB	297.571 ± 4.407	121.347 ± 0.938	130.673 ^b ± 0.951	157.265 ± 1.198
	BB	299.432 ± 5.071	121.324 ± 1.079	127.541 ^a ± 1.094	154.676 ± 1.379
24 months	AA	372.308 ± 11.380	125.923 ± 1.305	138.615 ^{ab} ± 2.081	168.923 ^{ab} ± 2.857
	AB	369.531 ± 5.861	126.633 ± 0.672	138.918 ^b ± 1.072	170.276 ^b ± 1.472
	BB	357.162 ± 6.745	125.541 ± 0.774	135.162 ^a ± 1.233	165.162 ^a ± 1.694

Note LSM in a column with no common superscripts differ significantly, low-case character represents significance at $P < 0.05$, capital character represents significance at $P < 0.01$. The same meaning in the following tables

Table 3 The LSM and SE between genotypes of the UCP-3/*BglI* polymorphism in Nanyang cattle

Growth stage	Genotypes	Body weight(kg)	Withers height(cm)	Body length(cm)	Heart girth(cm)
6 months	AA	159.476 ^{AB} ± 4.142	106.000 ^{ab} ± 1.151	105.429 ^{ab} ± 1.341	128.095 ± 1.709
	AB	162.103 ^B ± 2.493	106.793 ^b ± 0.693	106.448 ^b ± 0.807	129.328 ± 1.028
	BB	146.462 ^A ± 5.265	102.692 ^a ± 1.463	102.231 ^a ± 1.704	127.692 ± 2.172
12 months	AA	226.143 ± 5.268	113.714 ^{AB} ± 0.859	116.857 ± 1.770	141.524 ± 1.830
	AB	225.103 ± 3.170	114.534 ^B ± 0.517	117.690 ± 1.065	141.466 ± 1.101
	BB	217.462 ± 6.696	111.308 ^A ± 1.092	114.846 ± 2.249	140.231 ± 2.326
18 months	AA	299.692 ± 8.453	122.154 ± 1.818	129.692 ^{ab} ± 1.861	154.462 ± 2.311
	AB	297.571 ± 4.354	121.347 ± 0.936	130.673 ^b ± 0.959	157.265 ± 1.190
	BB	300.162 ± 5.010	121.378 ± 1.078	127.405 ^a ± 1.103	154.865 ± 1.370
24 months	AA	365.923 ± 11.403	127.923 ^b ± 1.125	138.231 ^{ab} ± 2.071	167.038 ^{ab} ± 2.724
	AB	370.551 ± 5.873	126.980 ^{ab} ± 0.580	139.408 ^b ± 1.067	170.918 ^b ± 1.403
	BB	363.595 ± 6.759	125.243 ^a ± 0.667	135.351 ^a ± 1.228	166.649 ^a ± 1.615

Note LSM in a column with no common superscripts differ significantly, low-case character represents significance at $P < 0.05$, capital character represents significance at $P < 0.01$. The same meaning in the following tables

($P < 0.01$ or $P < 0.05$) in Nanyang cattle at 6 months with genotype AB than those with genotype BB. The cattle with genotype AB had higher withers height at 12 months ($P < 0.01$) and body length at 18 months ($P < 0.05$) than those with genotype BB. At 24 months, the body length and heart girth were greater ($P < 0.05$) in Nanyang cattle with genotype AB than those with genotype BB, and the cattle with genotype AA had higher withers height ($P < 0.05$) than those with genotype BB.

Discussion

The *IGF-IR/TaqI* polymorphism is located in a noncoding region (intron) of the *IGF-IR* gene. Researchers concluded

that this polymorphism is not very useful in studies on the identification of quantitative trait loci (QTL) in cattle due to the absence of B allele in *B. taurus* and its low frequency in *B. indicus* [11, 15, 16]. In Girolando cows, frequencies of 0.83 for allele A and 0.17 for allele B were obtained, and significant association of genotypes AA and BB with higher milk production and of genotype BB with a short calving interval. Few data are available to date regarding the effect of this polymorphism on growth and carcass traits in beef cattle. Curia et al. [15] found no association between the genotypes of this polymorphism and growth or carcass traits of beef cattle. The present results showed this polymorphism had no significant effects on growth traits of Nanyang cattle, indicating the possibility that this polymorphism is not in linkage disequilibrium with QTL of

interest. This statement is in line with the review of Switonski [16] who reported that chromosome 21 is not one of the most interesting localization of loci related to growth and carcass composition in beef cattle.

The fusion of myoblasts is a key step in muscular differentiation, generating multinucleated muscle fibers from mononucleated myoblasts. Calpain, a ubiquitous cytoplasmic cysteine protease, maybe involved in cytoskeletal and extracellular matrix reorganization in myoblasts, thus plays a key role in myoblast fusion. M-calpain seems to be directly involved in early myoblast differentiation, because its mRNA appears before fusion and its activity increases all along myotube formation [5, 6]. Zhang et al. [12], Juszczuk-Kubiak et al. [13], Lara et al. [17] reported the existence of two alleles in *m-calpain/HhaI* polymorphism in different breeds. The frequencies of alleles and genotypes of *m-calpain/HhaI* polymorphism were distributed differently between breeds. The present results demonstrated association between the AB genotypes and superior growth traits of Nanyang cows at different growth stages. The *m-calpain/HhaI* polymorphism could be a potential marker for selection and breeding of Nanyang cattle.

UCP-3, one member of uncoupling protein family, involved in regulation of energy metabolism, has important physiological effects on economical important traits of farm animals. Bovine *UCP-3* gene is located in BTA15 [18]. Sherman et al. [19] observed significant association between a SNP (A/G) in bovine *UCP-3* gene intron 3 and average daily gain and partial efficiency of growth in Continental $\times$ British hybrid beef steers. Han [20] reported the existence of three SNPs (G/A, C/T and G/T) in exon 3 and four genotypes in Chinese Qinchuan cattle. Association studies showed that the genotype AA has higher slaughter weight, carcass weight, carcass length, eye muscle area, water holding capacity, and marbling than other genotypes, the genotype AB has higher back fat than other genotypes. Li [14] found the allele A of *UCP-3/BglII* polymorphism has higher frequencies than allele B in Nanyang, Luxi, and Yanbian cattle. Association analysis showed the superiority of the AA genotype over the AB genotype for the content of β -globin in Luxi cattle. The present results revealed significant association between the genotypes of *UCP-3/BglII* polymorphism and growth traits in Nanyang cattle, and genotype AB was superior over other genotypes, suggesting that this polymorphism is associated with loci affecting cattle growth.

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