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Full Length Research Paper

Association of polymorphisms in the *Pit-1* intron 5 with body measurements in Chinese Cattle

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The *Pit-1* gene was studied as a candidate for genetic markers of growth traits in Chinese cattle in this study. The single-strand conformation polymorphism method was used to identify polymorphism in the *Pit-1* intron 5. One single nucleotide polymorphism, *Pit*1I5, was found in intron 5. The frequencies of allele A of Nanyang cattle (NY), Qinchuan cattle (QC), Jiaxianhong cattle (JX), Luxi cattle (LX) and Holstein cow (H) populations were 0.444, 0.477, 0.538, 0.523 and 0.475, respectively. Associations of the polymorphisms with growth traits in Nanyang cattle were analyzed using a general linear model procedure. The following parameters were greater in individuals with AA genotype than with AB genotype: body weight, average day gain, body height, chest girth at six, 12, 18 and 24 months (P<0.01), respectively. Body weight and body size also showed a trend of allele A > allele B in other age groups. Therefore, genotype AA maybe a dominant genotype and allele A may be a dominant allele. These results imply that allele A of *Pit-1* gene may likely affect growth traits positively.

Key words: Beef cattle, growth traits, *Pit-1* intron 5, polymorphism.

INTRODUCTION

Growth and carcass traits, which are under the control of multiple genes, are economically important traits in livestock. Selection of animals with higher growth rate and better carcass composition is of great significance to breeders and consumers. Current technologies enable scientists to improve on the accuracy and efficiency of traditional selection methods by applying genetic markers through marker-assisted selection. Therefore, genetic polymorphisms (marker loci) that are significantly associated with certain traits of interest are very useful (Castendyk and Webster, 2007).In this study, *Pit*-1 was examined as a genetic marker candidate gene. It is a pituitary-specific transcription factor that is responsible for pituitary development and hormone expression in mammals. It was shown to control transcription of the growth hormone, prolactin, the thyroid-stimulation hormone, [beta]-subunit, the growth hormone receptor hormone (GHRH) receptor genes, and the *Pit*-1 gene itself (Zhao, 2004).

Mutations in the *Pit-1* gene lead to the absence of growth hormone and to pituitary hypoplasia in mice and to congenital hypothyroidism, dwarfism and prolactin deficiency in humans. In pigs, *Pit*-1 was found to be related to birth weight, weaning weight and average daily gain (ADG) (Yu et al., 1995). Also in pigs, associations were discovered with backfat, as well as lean-to-fat ratio. In cattle, *Pit-1* was found to be associated with body composition and milk yield (Renaville et al., 1997). In bovine,

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Abbreviations: NY, Nanyang cattle; **QC,** Qinchuan cattle; **JX,** Jiaxianhong cattle; **LX,** Luxi cattle; **H,** Holstein cow.

HinfI polymorphism had been researched at Pit-1 locus (Woollard et al., 1994; Renaville et al., 1997), and also the *Pit-1* gene polymorphism with the relationship with milk yield, conformation traits in Italian Holstein-Friesian bulls (Renaville et al., 1997 a). Xue (2006) applied polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to analyze the effect of the genetic variations of the POU1F1 gene on growth traits of 100 Nanyang cattle. Recently, the Pit-1/ HinfI and Pit-1/ Taq I loci using PCR-RFLP approach in a sample of 308 Podolica young bulls was reported (Selvaggi et al., 2011). Though there is little research about different species in Chinese cattle, the current study was designed to screen the *Pit-1* gene intron 5 for polymorphisms and to analyze the association of these polymorphisms with growth traits in Chinese indigenous cattle.

MATERIALS AND METHODS

Animals and management

Blood samples were obtained from 623 individuals of four Chinese cattle breeds: Nanyang (NY, n = 232), Qinchuan (QC, n = 65), Jiaxian (JX, n = 143), Luxi (LX, n = 122) and Chinese Holstein (CH, n = 61). The test population of NY and QC cattle was reared in the Henan Nanyang Cattle Conservation Farm and Shaanxi Qinchuan Cattle Conservation Farm of China, respectively. The cattle in the conservation farm were under the same nutrition conditions. The test population of JX cattle was dispersed in the village in Henan province of China. The body measurements (including body height, body length and heart girth) of 100 NY cattle were collected at six, 12, 18 and 24 months, respectively. These body measurements of 143 JX and 61 QC cattle were collected too, which were all more than two years old. Several milk traits (milk fat rate, milk protein rate and 305 ds milk yield) of the 61 CH cattle were collected in the dairy cattle farm.

Genotype determination

Total genomic DNA was isolated from muscle tissue and whole blood using the normal phenol-chloroform extraction method (Zhang et al., 2007). The *pit-1* intron 5 was amplified using the primers (forward primer 5′-CCT CTG TCC ATG GGA TTT C-3', reverse primer 5′-AAA TGT CCC C A GAA CTC AG -3′) designed by Zhao et al. (2004). PCR was carried out in 25 µl reaction volume containing 20 ng of DNA, 0.4 µM of each primer, 50 µM of each dNTP (Tiangen Biotech Co., Ltd, China), 0.6 U of Taq DNA polymerase (Sino-American Biotechnology Co., China), 1×PCR buffer (10 mM Tris-HCl [pH 9.0 at 25°C]; 1.5 mM $MgCl_2$; 50 mM KCl; 0.1% Triton X-100). PCR was carried out on a PTC-200 thermocycler (MJ Research Inc.) under the following conditions: 97°C for 2 min, followed by 35 cycles of 95°C for 45 s, 63°C for 1 min, and 72°C for 1 min. After 35 cycles, reactions were finished by an extension of 5 min at 72°C described by Zhao et al. (2004).

Single strand conformation polymorphism (SSCP) method was used to screen for mutations within the amplified region. The reaction mixture, which included 10 µL of digested PCR product, 10 µL of ddH_2O and 12 µL of loading dye, was denatured at 95°C for 5 min, and placed in ice for 10 min. The samples were then loaded on 10% nondenaturing polyacrylamide gels, with 10% urea or 10% formamide to improve the resolution of the DNA bands on the gel.

Statistical analysis

The genotypic and allelic frequencies, heterozygosities (h), polymorphism information content (PIC) and effective number of alleles (Ne) were calculated by using the methods reported Nei and Roychoudhurg, (1974). The Chi-square (χ^2) test was used to check whether the populations were in Hardy-Weinberg equilibrium or not. Associations of the animal genotypes with growth traits were determined by analysis of variance of quantitative traits, which included birth weight, withers height, body length, heart girth, hucklebone width, average day gain at six, 12, 18 and 24 months using General Linear Model (GLM) procedures in SPSS (SPSS 13.0, corp inc.). Linkage disequilibrium of the SNPs and haplotype frequencies were estimated through PowerMarker (Liu and Muse, 2005). The general linear model was used to evaluate the association between the genotypes and haplotypes of *Pit-1* and the body measurements of cattle. Independent variables were *Pit-1* genotype (or haplotype), breed, age and sex. Statistical model was displayed as follows:

$$Yijklm = u + Gi + Bj + Ak + Sl + eijklm$$

Where, Yijklm, observed value; u, overall mean for each trait; Gi, fixed effect associated with ith genotype; Bj, fixed effect associated with jth breed; Ak, fixed effect associated with kth age; Sl, fixed effect associated lth sex; eijkml, random error.

The least squares means (LSM) of the GLM were used to determine the level of significance in pairwise comparisons between genotype classes. LSM also known as population marginal means, are the values for class means after adjustment for all covariates in the model (Al-Shali et al., 2004). Pairwise comparisons of LSMs were performed using Fisher's protected least significant difference (LSD) t-test procedure.

RESULTS

Genetic polymorphism of the *Pit-1* gene and χ^2 test

χ^2 test showed that the polymorphism of the *Pit-1* locus in the population of NY and H was not at Hardy-Weinberg equilibrium. The genetic diversity of the locus was then calculated. Table 1 shows that The frequencies of genotype AA/BB of NY, QC, JX, LX, and H populations were 0.444/0.556, 0.477/0.523, 0.538/0.462, 0.523/0.477, 0.475/0.525 respectively. For NY, QC, JX, LX, and H populations, the gene heterozygosity(Hk)/ effective allele gene number(Ne)) were 0.494/1.975, 0.499/1.996, 0.497/1.988, 0.499/1.996, 0.499/1.995 respectively. These results show that the population had high heterozygosity and high poly-morphism information content, suggesting that it had a high level of genetic variation and information content.

Effects of *Pit-1* intron 5 polymorphism on the growth traits of Chinese cattle

Genotypes had a significant effect on birth weight, body weight and body length at six months, and body weight, body height, body length and chest girth at 12 months (P<0.05). The linear model was then applied to analyze

Table 1. Genotypic frequencies in the *Pit-1* intron 5 and the statistical test results for Hardy-Weinberg equilibrium in the cattle breeds.

Breed	N	P_{AA}	P_{BB}	Jk	Hk	Ne	x^2
Nanyang	232	0.444	0.556	0.506	0.494	1.975	**35.08
Qinchuan	65	0.477	0.523	0.501	0.499	1.996	2.56
Jiaxianhong	143	0.538	0.462	0.503	0.497	1.988	0.27
Luxi	122	0.523	0.477	0.501	0.499	1.996	2.89
Holstien	61	0.475	0.525	0.501	0.499	1.995	**39.31

*, P<0.05; **, P<0.01.

Table 2. Least square analysis between *Pit-1* intron 5 and physical measure of Chinese Nanyang cattle.

Age	Growth trait	AA	AB	BB
Six months	Body weight (kg)	169.816±4.441^{Aa}	150.100±4.998^{Bb}	158.703±4.500^{b}
	Withers height (cm)	108.684±7.928^{Aa}	102.800±8.923^{Bb}	106.270±8.034^{b}
	Body length (cm)	109.079±1.013^{Aa}	102.067±1.140^{Bb}	105.135±1.027^{b}
	Heart girth (cm)	132.921±1.138^{Aa}	124.500±1.280^{Bb}	128.541±1.153^{b}
	Hucklebone width (cm)	18.605±0.281	18.138±0.316	18.216±0.285
	Average day gain(kg)	0.771±0.029^{Aa}	0.671±0.033^{Bb}	0.718±0.029^{b}
Twelve months	Body weight (kg)	228.237±4.441	220.367±4.998	220.432±4.500
	Withers height (cm)	115.053±7.928	146.233±8.923	114.162±8.034
	Body length (cm)	120.263±1.013^{Ab}	114.233±1.140^{Ba}	116.216±1.027^{a}
	Heart girth (cm)	145.605±1.138^{A}	136.667±1.280^{B}	140.892±1.153^{B}
	Hucklebone width (cm)	21.274±0.281^{Ab}	20.250±0.316^{Ba}	20.500±0.285^{a}
	Average day gain (kg)	0.325±0.029^{A}	0.390±0.033^{B}	0.343±0.029^{AB}
Eighteen months	Body weight (kg)	304.474±4.441	293.867±4.998	295.703±4.500
	Withers height (cm)	121.711±7.928	119.967±8.924	122.189±8.034
	Body length (cm)	130.053±1.013^{A}	125.633±1.140^{B}	128.838±1.027^{B}
	Heart girth (cm)	160.842±1.138^{A}	150.600±1.280^{B}	155.622±1.153^{B}
	Hucklebone width (cm)	23.605±0.281^{A}	22.700±0.316^{B}	23.041±0.285^{AB}
	Average day gain (kg)	0.424±0.029	0.408±0.033	0.418±0.029
Two years	Body weight (kg)	392.842±4.441^{A}	347.9000±4.998^{B}	358.568±4.500^{B}
	Withers height (cm)	127.184±7.98^{A}	124.400±8.923^{B}	127.189±8.034^{A}
	Body length (cm)	142.737±1.013^{A}	132.233±1.140^{A}	137.973±1.027^{B}
	Heart girth (cm)	176.132±1.138^{A}	159.600±1.280^{AC}	169.257±1.153^{B}
	Hucklebone width (cm)	26.461±0.281^{A}	24.183±0.316^{B}	25.176±0.285^{B}
	Average day gain (kg)	0.491±0.029^{A}	0.300±0.033^{B}	0.349±0.029^{B}

Data with a different letters (A, B, C) and (a, b, c) within the same line differ significantly at P<0.01 and 0.01<P<0.05, respectively.

the birth season effect, genotype effect and the cooperative effect of the two factors. The growth traits (body weight, withers height, body length, heart girth, hucklebone width and average day gain) at every age were analyzed, and the results are shown in Table 2.Table 2 shows the following results: (1) comparing with AB and BB individuals, AA individuals had higher body weight, withers height, body length, heart girth, hucklebone width and average day gain at six months (*P*<0.01) and had higher body weight, body length, heart girth, hucklebone width at 12 months, it also had a higher body weight, body length, heart girth, hucklebone width and average day gain in 18 months and two years old (*P*<0.01); (2) AB individuals only had higher withers height and and average day gain at 12 months when compared with BB individuals. Although, there was no significant difference with respect to other parameters, there was always a trend towards favoring allele A; (3)

there was no significant difference between the individuals with genotype AB and the individuals with genotype BB ($P>0.05$).

Table 2 shows that in body length, heart length, body weight, hucklebone width, body height and average day gain, individuals with genotype AA in NY population were higher than that of genotype AB and BB in NY population, except withers height, average day gain in 12 months and withers height in 18 months and two years.

DISCUSSION

Results show that age had a significant effect on growth traits ($P<0.05$), and that the cooperative effect between age and genotype was significant to the growth traits of Chinese cattle at every age ($P<0.05$). So we could conclude that genotypes were the main reason for the diversity of the growth traits in Chinese cattle.

The polymorphism in *Pit1E6* in exon 6 was detected by Woollard et al. (1994). Previous studies of this polymorphism in Italian Holstein-Friesian bulls revealed that allele A had a positive effect on milk yield traits, body depth, angularity and rear leg set (Renaville et al., 1997a). The same authors also found a relationship of allele B with higher body weight at seven months of age in double-muscled Belgian Blue bulls. Allele A was shown to have more desirable daily milk yield and milk composition in Polish Black-and-White cows (Zwierzchowski et al., 2002). However, Di Stasio et al. (2002) found no association of the genotypes with meat production traits in Piemontese cattle. In addition, Zwierzchowski et al. (2001) found no relationship of this marker with growth and carcass traits in beef cattle. Zhao et al. (2004) found no significant association of this polymorphism with growth and carcass traits in Angus beef cattle.

Our results show that the allele A is associated with better growth traits of NY cattle, but for the withers height, genotype AA was lower than genotype BB and AB. It may suggest that there are either multiple mutations or levels of LD in different ages and populations and species. The association of the *Pit-1* polymorphism with growth traits of Chinese cattle revealed from this study suggests its feasibility as a molecular breeding marker. For the recent research, new evidence suggests that abundant small RNAs, microRNAs (miRNAs) act as regulatory vehicles to repress translation or cleave RNA transcripts, depending on their complementarity to the target gene, and result in modulation of gene expression. Ying et al. (2004) showed that the miRNAs derived from introns can suppress intracellular RNA homologues and regulate the gene function. It could be suggested that the intron 5 of *Pit-1* in cattle may play important role in the cattle's growth development. Our research show that individuals with AA genotype were greater than that with AB genotype in body weight, average day gain, body height, chest girth at six, 12, 18 and 24 months.

This information could help animal scientists to develop genetic markers or biomarkers in cattle breeding. According to the biological function of the *Pit-1*, it is worthy to investigate the associations between these genotype and the meat quality traits in the next study.

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