Association Of Polymorphisms In The *Pit-1* Intron 5 With Growth Traits In Chinese Cattle

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Introduction

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 GHRH receptor genes, and the *Pit-1* gene itself (Zhao,et al.,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 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). The current study was designed to screen the *Pit-1* gene for polymorphisms and to analyze the association of these polymorphisms with growth traits in Chinese cattle.

Materials and Methods

Experimental Animals. One mature female population of Nanyang cattle (NY), from the breeding center of Nanyang cattle in Nanyang, Henan province of China was used for this study. Blood samples from 232 cattle were collected into an anticoagulant ACD (0.48% citric acid, 1.32% sodium citrate, 1.47% glucose) (6:1), and stored at －80 ℃ for DNA preparation; 61 Chinese Holstein cows(H) blood samples were collected from Shaanxi Caotan livestock farm;143 JiaxianRed cattle(JXR)blood samples, 65 Qinchuan cattle(QC) blood samples, 22 Luxi cattle(LX) blood samples and 19 Xizhen cattle(XZ) blood samples were kept in Shaanxi key laboratory of molecular biology for agriculture; total of 542 cattle were used and genotyped for the *pit-1* intron 5 polymorphism in this study.

Genotype Determination. Total genomic DNA was isolated from muscle tissue and whole blood using the normal phenol-chloroform extraction method (Sambrook, et al., 1989). The *pit-1* intron 5 was amplified using the primers(forward primer 5′-CCT CTG TCC ATG GGA TTT C-3’ -3′, reverse primer 5′-AAA TGT CCC CCA GAA CTC AG -3′) designed and PCR

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was carried out by Zhao et al. 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. We calculated the genotypic and allelic frequencies, heterozygosities (h), polymorphism information content (PIC) and effective number of alleles (Ne) by using the methods reported by (Guo et al., 2002)., and (Nei et al., 1974). 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; twelve; eighteen; twenty four months using GLM procedures in SPSS (version 13.0). The linearity model was applied to analyze the association of variations of ***pit-1*** gene with growth traits of Nanyang cattle.Yij =μ+ marker + age + ma +marker/age + eij;Where Yij is the observation of the growth traits, μ is the overall mean for each trait, marker is the effect of genotype, age is the effect of age, ma is the cooperative effect of age and genotype and eij is the random residual effect. After the least-squares estimation to the growth traits of Chinese cattle with different genotypes in ***pit-1*** intron 5 locus was generate, SPSS (version 13.0), was applied to for effect analysis of ANOVA.

Results and Discussions

Genetic polymorphism of the *pit-1* gene and χ2 test. χ2 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 showed that the frequencies of allele A/B of NY, QC, JXR, XZ, LX and H populations were 0.444/0.556, 0.477/0.523, 0.538/0.462, 0.421/0.579, 0.523/0.477, 0.475/0.525 respectively. These results showed that the population had high heterozygosity and high polymorphism information content, suggesting that it had a high level of genetic variation and more genetic information.

Effect analysis of ANOVA on the growth traits of Chinese Cattle. Effect analysis of ANOVA showed that age had a significant effect on body traits ($P<0.05$). Genotypes had a significant effect on birth weight, body weight and body length at 6 months, and body weight, body height, body length and chest girth at 12 months ($P<0.05$). The linearity model was then applied to analyze the birth season effect, genotype effect and the cooperative effect of the two factors. The growth traits (body weight, body height, body length and chest girth) at every age were analyzed, and the results are shown in tables I. Table I indicates the following results (1) comparing to AB and BB individuals, AA individuals had higher body weight, body height, body length, chest girth, average day gain at 6 months ($P<0.01$) and had higher body length and chest girth, hucklebone width at 12 months and 18 months ($P<0.01$); (2) BB individuals only had higher body length and chest girth at 24 months when compared to AB 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 I shows that in body length, heart length, body weight, hucklebone width, body height, and average day gain individuals with genotype AA in Nanyang population were higher than that of genotype AB and BB in Nanyang population except body height, average day gain in 12 months and hucklebone width, average day gain in 18 months

Table 1 Least square analysis between polymorphism of *pit-1* intron 5 and growth traits in Chinese Nanyang Cattle

Growth traits	AA	AB	BB
Body weight at six months (kg)	169.816±4.441^{Aa}	150.100±4.998^{Bb}	158.703±4.500^{b}
Withers height at six months (cm)	108.684±7.928^{Aa}	102.800±8.923^{Bb}	106.270±8.034^{b}
Body length at six months (cm)	109.079±1.013^{Aa}	102.067±1.140^{Bb}	105.135±1.027^{b}
Heart girth at six months (cm)	132.921±1.138^{Aa}	124.500±1.280^{Bb}	128.541±1.153^{b}
Hucklebone width at six months (cm)	18.605±0.281	18.138±0.316	18.216±0.285
Average day gainat six months (kg)	0.771±0.029^{Aa}	0.671±0.033^{Bb}	0.718±0.029^{b}
Body weight at 12 months (kg)	228.237±4.441	220.367±4.998	220.432±4.500
Withers height at 12 months (cm)	115.053±7.928	146.233±8.923	114.162±8.034
Body length at 12 months (cm)	120.263±1.013^{Ab}	114.233±1.140^{Ba}	116.216±1.027^{a}
Heart girth at 12 months (cm)	145.605±1.138^{A}	136.667±1.280^{B}	140.892±1.153^{B}
Hucklebone width at 12 months (cm)	21.274±0.281^{Ab}	20.250±0.316^{Ba}	20.500±0.285^{a}
Average day gain at 12 months (kg)	0.325±0.029^{A}	0.390±0.033^{B}	0.343±0.029^{AB}
Body weight at 18 months (kg)	304.474±4.441	293.867±4.998	295.703±4.500
Withers height at 18 months (cm)	121.711±7.928	119.967±8.924	122.189±8.034
Body length at 18 months (cm)	13.053±1.013^{A}	125.633±1.140^{B}	128.838±1.027^{B}
Heart girth at 18 months (cm)	160.842±1.138^{A}	150.600±1.280^{B}	155.622±1.153^{B}
Hucklebone width at 18 months (cm)	23.605±0.281^{A}	22.700±0.316^{B}	23.041±0.285^{AB}
Average day gain at 18 months (kg)	0.424±0.029	0.408±0.033	0.418±0.029
Body weight at 24 months (kg)	392.842±4.441^{A}	347.9000±4.998^{B}	358.568±4.500^{B}
Withers height at 24 months (cm)	127.184±7.98^{A}	124.400±8.923^{B}	127.189±8.034^{A}
Body length at 24 months (cm)	142.737±1.013^{A}	132.233±1.140^{A}	137.973±1.027^{B}
Heart girth at 24 months (cm)	176.132±1.138^{A}	159.600±1.280^{AC}	169.257±1.153^{B}
Hucklebone width at 24 months (cm)	26.461±0.281^{A}	24.183±0.316^{B}	25.176±0.285^{B}
Average day gain at 24 months (kg)	0.491±0.029^{A}	0.300±0.033^{B}	0.349±0.029^{B}

Notes: Data with a different letter (A,B,C) and (a,b,c) within the same line differ significantly at $P<0.01$ and $0.01<P<0.05$, resepectively.

Results showed 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 Pit1E6 in exon 6 was detected (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., 1997). The same authors also found a relationship of allele B with higher body weight at 7 month 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. Q Zhao found no significant association of this polymorphism with growth and carcass traits in Angus beef cattle.

Conclusion

Allele B of *pit-1* should be associated with better growth traits of Nanyang cattle. 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. In this study, cattle with genotype AA had remarkable growth predominance, and some of them, which had better performance, could be used to the breeding of new breeds of beef cattle. Meanwhile, this study also suggests strengthening the improvement of Chinese cattle. These suggestions may be instructional for early breeding selection.

Therefore, the effects of this genetic marker have varied from study to study, which could be due to different statistical models used, different numbers of animals genotyped, or genetic composition of the breeds studied. More animals need to be studied to better understand the effect of this marker on production traits in cattle.

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