

## Relationship of Pit-1 polymorphisms with growth traits in Chinese Cattle

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**Abstract**—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, Pit115, was found in intron 5. The frequencies of alleles A/B of Nanyang cattle(NY), Qinchuan cattle(QC), Jiexianhong cattle(JXH), Xizhen cattle(XZ), Luxi cattle(LX) and Holstein cow(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. 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 an AA genotype than with an AB genotype: body weight, average day gain, body height, chest girth at 6, 12, 18 and 24 months ( $P < 0.01$ ). Body weight and body size also showed a trend of allele B > allele A in other age groups. Therefore, genotype BB maybe a dominant genotype and allele B may be a dominant allele. These results imply that allele B of Pit-1 gene likely positively affects growth traits.

**Keywords**- Beef Cattle; Growth; Traits; Pit-1 intron 5; Polymorphism

### I. 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.

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 GHRH receptor genes, and the Pit-1 gene itself [1].

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 [2]. 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 [3]. 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.

### II. MATERIALS AND METHODS

#### A. 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  $-80^{\circ}\text{C}$  for DNA preparation; 61 Chinese Holstein cows(H) blood samples were collected from Shaanxi Caotan livestock farm; 143 Jiexianhong cattle(JXH) 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.

### B. Genotype Determination

Total genomic DNA was isolated from muscle tissue and whole blood using the normal phenol-chloroform extraction method [4]. 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 by Zhao et al[1]. PCR was carried out in 25  $\mu\text{L}$  reaction volume containing 20 ng of DNA, 0.4  $\mu\text{M}$  of each primer, 50  $\mu\text{M}$  of each dNTP(Tiagen Biotech Co., Ltd, China), 0.6 U of Taq DNA polymerase(Sino-American Biotechnology Co., China), 1 $\times$ PCR buffer(10 mM Tris-HCl [pH 9.0 at 25  $^{\circ}\text{C}$ ]; 1.5 mM  $\text{MgCl}_2$ ; 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  $^{\circ}\text{C}$  for 2 min, followed by 35 cycles of 95  $^{\circ}\text{C}$  for 45 s, 63  $^{\circ}\text{C}$  for 1 min, and 72  $^{\circ}\text{C}$  for 1 min. After 35 cycles, reactions were finished by an extension of 5 min at 72  $^{\circ}\text{C}$  described by Zhao et al. [1].

Single-strand conformation polymorphism (SSCP) method was used to screen for mutations within the amplified region. The reaction mixture, which included 10  $\mu\text{L}$  of digested PCR product, 10  $\mu\text{L}$  of ddH $_2$ O, and 12  $\mu\text{L}$  of loading dye, was denatured at 95 $^{\circ}\text{C}$  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.

### C. 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., [5] and Nei et al. [6].

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.

$$Y_{ij} = \mu + \text{marker} + \text{age} + \text{ma} + \text{marker}/\text{age} + e_{ij}$$

Where  $Y_{ij}$  is the observation of the growth traits,  $\mu$  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  $e_{ij}$  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.

## III. RESULTS

### A. Genetic polymorphism of the pit-1 gene and $\chi^2$ test

$\chi^2$  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 showed that the frequencies of allele A/B of NY, QC, JXH, 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.

Table I

### B. 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 2. Table 2 shows 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 2 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 II

## IV. DISCUSSIONS

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 by Woollard et al. [7]. 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 [8]. 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 [9]. However, Di Stasio et al. [10] (2002) found no association of the genotypes with meat production traits in Piemontese cattle. In addition, Zwierzchowski et al. [11] (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.

Our results showed that the allele B 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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#### REFERENCES

- [1] Zhao Q., Davis M. E., Hines H. C. , Associations of polymorphisms in the Pit-1 gene with growth and carcass traits in Angus beef cattle, *Journal of Animal Science* 82 (2004) 2229-2233.
- [2] Yu T. P., Tuggle C. K., Schmitz C. B., M. F. Rothschild, Association of Pit-1 polymorphism with growth and carcass traits in pigs, *J. Anim. Sci.* 73 (1995) 1282-1288.
- [3] Renaville R., Gengler N., Vrech E., Prandi A., MassartS., Corradini ., Bertozzi C., Mortiaux F., Burny A., Portetelle D., Pit-1 gene polymorphism, milk yield, and conformation traits for Italian Holstein-Friesian bulls, *J. Dairy Sci.* 80 (1997 a) 3431-3438.
- [4] Sambrook J., Fritsch E.F., Maniatis T., *Molecular Cloning: A Laboratory Manual*, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.
- [5] Guo M-C. Research on the information model of population genetic variation [C]. Doctoral dissertation. Northwest Sci-Tech University of Agriculture and Forestry, 2002
- [6] Nei M., Roychoudhurg A. K., Sampling variance of heterozygosity and genetic distance, *Genetics* 76 (1974) 379-390.
- [7] Woollard J., Schmitz C. B., Freeman A. E., Tuggle C. K., Rapid communication: Hinfl polymorphism at the bovine Pit-1 locus, *J. Anim. Sci.* 72 (1994) 3267.
- [8] Renaville R., Gengler N., Parmentier I., Mortiaux F., Massart S., Bertozzi C., Burny A., Portetelle D., Pit-1 gene Hinfl RFLP and growth traits in double- muscled Belgian Blue Cattle, *J. Anim. Sci.* 75 (1997b) (Suppl. 1)146. (Abstr.)
- [9] Zwierzchowski L., Krzyzewski J., Strzalkowska N., Siadkowska E., Ryniewicz A., Effect of polymorphisms of growth hormone (GH), Pit-1, and leptin (LEP) genes, cow's age, lactation stage and somatic cell count on milk yield and composition of Polish Black-and-White cows, *Anim. Sci. Papers Reports, Inst. Genet.Anim.Breed., Jastrzebiec, Poland.* 20 (2002) 213-227.
- [10] Di Stasio L., Saratore S., Alberta A., Lack of association of GH1 and Pou1f1 gene variants with meat production traits in Piemontese cattle, *Anim. Genet.* 33 (2002) 61-64.
- [11] Zwierzchowski L., Oprzydek J., Dymnicki E., Dzierzbicki P., An association of growth hormone, [kappa]-casein, [beta]-lactoglobulin, leptin and Pit-1 loci polymorphism with growth rate and carcass traits in beef cattle, *Anim. Sci. Papers Reports, Inst. Genet. Anim. Breed., Jastrzebiec, Poland.* 19 (2001) 65-78.

TABLE I. GENOTYPIC FREQUENCIES OF POLYMORPHISMS

gene	breeds	N	PAA	PBB	Jk	Hk	Ne	PIC	x^2
PIT-5	NY (Nanyang)	232	0.444	0.556	0.506	0.494	1.975	0.372	**35.08
	QC (Qinchuan)	65	0.477	0.523	0.501	0.499	1.996	0.374	2.56
	JXH (Jiaxianhong)	143	0.538	0.462	0.503	0.497	1.988	0.374	0.27
	XZ (Xizhen)	19	0.421	0.579	0.512	0.488	1.951	0.369	2.36
	LX (Luxi)	22	0.523	0.477	0.501	0.499	1.996	0.374	2.89
	H (Holstien)	61	0.475	0.525	0.501	0.499	1.995	0.374	**39.31

TABLE II. LEAST SQUARE ANALYSIS BETWEEN PIT-1 INTRON 5 AND PHYSICAL MEASURE OF CHINESE NANYANG CATTLE

Age	Growth traits	AA	AB	BB
Six months	Body weight(kg)	169.816±4.441Aa	150.100±4.998Bb	158.703±4.500b
	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±1.013Ab	114.233±1.140Ba	116.216±1.027a
	Heart girth(cm)	145.605±1.138A	136.667±1.280B	140.892±1.153B
	Hucklebone width(cm)	21.274±0.281 Ab	20.250±0.316 Ba	20.500±0.285 a
	Average day gain(kg)	0.325±0.029A	0.390±0.033B	0.343±0.029AB
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)	13.053±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±0.281A	22.700±0.316B	23.041±0.285AB
	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±7.98A	124.400±8.923B	127.189±8.034A
	Body length(cm)	142.737±1.013A	132.233±1.140A	137.973±1.027B
	Heart girth(cm)	176.132±1.138 A	159.600±1.280 AC	169.257±1.153 B
	Hucklebone width(cm)	26.461±0.281A	24.183±0.316B	25.176±0.285B
	Average day gain(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  $0.01 < P < 0.05$ , respectively.