A *Dde*I PCR-RFLP detecting a novel missense mutation of the *POU1F1* gene showed no effects on growth traits in cattle

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ABSTRACT: In this study, a novel missense (NM_174579:c.1201C>T) mutation in exon 6 at the bovine *POU1F1* locus is reported, which results in p.S284F, namely, Ser (TCT) > Phe (TTT) at position 284 of the mature protein. A *Dde*I PCR-RFLP was used to determine the genotypes. The polymorphism was studied in eight Chinese cattle breeds (Nanyang, n = 251; Qinchuan, 149; Jiaxian Red, 144; Chinese Holstein, 61; Luxi, 57; Angus, 49; Jinnan, 60; Guyuan, 192). The frequencies of the *POU1F1 T* allele in the analyzed populations ranged from 0.010 to 0.053. The relationships between the *Dde*I polymorphism and growth traits and body sizes were analyzed by adjusted linear model in 251 Nanyang cattle. Fixed effects of marker genotype, birth year, season of birth (spring vs. fall), age of dam, sire, farm and sex, and random effects of an animal were included. Statistical evaluation revealed no significant relationships between this polymorphism and birth weight, body weight and average daily gain for different growth periods (6-month old, 12-month old, 18-month old and 24-month old) body height, body length, heart girth and height at the hip cross for different growth periods (P > 0.05).

Keywords: bovine; *POU1F1* gene; PCR-RFLP; growth traits

POU1F1 (also named PIT-1 or GHF-1) is a member of the POU-domain family of transcription factors mainly expressed in the pituitary. Its expression is necessary for the normal differentiation, development and survival of three adenohypophysis cell types (thyrotrophs, somatotrophs and lactotrophs). It is also an important regulator for expression of growth hormone (GH), prolactin (PRL) and thyroid-stimulating hormone β (TSH-β) in mammals. Hence, *POU1F1* mutations may result in different expression of *GH*, *PRL*, *TSH*, and *POU1F1* gene itself (Li et al., 1990; Cohen et al., 1996). In mammals, *POU1F1* mutations have been found to be associated with mice Snell dwarf and Jackson dwarf,

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and also result in human dwarfish (Li et al., 1990; Pfaffle et al., 1992). In domestic animals, cattle, sheep and goat, the *POU1F1* gene was located on 1q21-q22 (Woollard et al., 2000), and in porcine on 13q46. According to Yu et al. (1995), Renaville et al. (1997a,b), Stančeková et al. (1999), Sun et al. (2002), and Zhao et al. (2004), genetic variations of cattle and porcine *POU1F1* gene were associated with economic traits and production performance. Moreover, QTL detection revealed that the region surrounding *POU1F1*, on cattle 1q21-q22 had an effect on animal production (Woollard et al., 2000). So, *POU1F1* gene is a potential candidate gene for growth traits.

The bovine *POU1F1* transcription unit is organized in 6 exons coding for a 291 AA (Amino Acid) polypeptide chain. In 1994, the silent mutation was revealed in exon 6 of the bovine *POU1F1* gene by a *Hin*fI PCR-RFLP (Woollard et al., 1994). Associations of this polymorphism with body composition and milk yield in dairy cattle, early-age body weight in beef cattle, carcass and growth traits in meat cattle, were described (Renaville et al., 1997a,b; Zhao et al., 2004; Xue et al., 2006). In 2004, two polymorphisms (*Hin*fI and *Nla*III PCR-RFLPs), one polymorphism (*Bst*NI PCR-RFLP) and one SNP were found in intron 3, intron 4 and intron 5 of the bovine *POU1F1* gene, respectively (Zhao et al., 2004).

In this paper, we report the identification of a novel missense mutation at the bovine *POU1F1* locus and describe a method based on a *Dde*I PCR-RFLP for its detection. Moreover, the effects of the polymorphism on growth traits are evaluated.

MATERIAL AND METHODS

DNA samples

Genomic DNA samples were obtained from 963 individuals belonging to eight cattle breeds: Nanyang (NY, n = 251), Qinchuan (QC, n = 149), Jiaxian Red (JX, n = 144), Chinese Holstein (CH, n = 61), Luxi (LX, n = 57), Angus (AN, n = 49), Jinnan (JN, n = 60) and Guyuan (also called Zaosheng; GY, n = 192). They represent the main breeds of China, reared in the provinces of Henan, Shaanxi, Shandong, Shanxi and Ningxia. A total of 6 275 records of growth traits and body sizes for different growth periods (6-month old, 12-month old, 18-month old and 24-month old) in 251 Nanyang cattle (NY) were collected for statistical analysis. DNA samples were extracted from leukocytes and tissue samples according to Mullenbach et al. (1989).

PCR-SSCP and DNA sequencing

A pair of primers from exon 6 and its flanking region of the cattle POU1F1 gene (forward: 5'-AAACCATCATCTCCCTTCTT-3'; reverse: 5'-AATGTACAATGTGCCTTCTGAG-3') designed by Woollard et al. (1994) was used for PCR amplification of a 451 bp fragment. The 25 µl PCR reaction contained 50 ng bovine genomic DNA, 0.5µM of each primer, 1X buffer (including 1.5mM MgCl2), 200µM dNTPs and 0.625 units of Taq DNA polymerase (MBI, Vilnius, Lithuania). The cycling protocol was 4 min at 95°C, 35 cycles of 94°C for 45 s, 53.5°C annealing for 45 s, 72°C for 1 min, with a final extension at 72°C for 10 min.

Aliquots of 5 µl of PCR products were mixed with 5 µl denaturing solution (95% formamide, 25mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C and chilled on ice (Orita et al., 1989). Denatured DNA was subjected to 10% PAGE (80 × 73 × 0.75 mm) in 1X TBE buffer and constant voltage (200 V) for 2.5–3.0 h. The gel was stained with 0.1% silver nitrate (Orita et al., 1989). The different PCR fragments from polymorphic SSCP patterns in cattle were cloned in T-vector (Promega, Wisconsin, USA) and sequenced in both directions using an ABI 3 730 DNA sequencer (Invitrogen, California, USA). These sequences were submitted to the GenBank database (accession numbers EF090615–EF090618). A novel mutation was detected and could be genotyped with restriction enzyme DdeI.

*Dde*I PCR-RFLP

Aliquots of 20 µl PCR products were digested with 15 U of DdeI (Toyobo, Osaka, Japan) for 5 h at 37°C following the supplier's instructions. The digested products were analyzed in 10% PAGE or in 3.0% agarose gel electrophoresis.

Statistical analysis

Statistical analysis was performed on records of growth traits of 251 Nanyang cattle (NY, n = 251). A Bonferroni correction (the Multiple Trait

Table 1. Genotype distribution and allelic frequencies at the bovine *POU1F1* locus

Breeds	Observed genotypes			Allelic frequencies		
	CC	*CT*	total	*C*	*T*	χ^2 (H.-W. E.)[1]
Nanyang (NY)	225	26	251	0.948	0.052	0.749
Qinchuan (QC)	139	10	149	0.966	0.034	0.180
Jiaxian Red (JX)	134	10	144	0.965	0.035	0.186
Chinese Holstein (CH)	59	2	61	0.984	0.016	0.017
Luxi (LX)	51	6	57	0.947	0.053	0.176
Angus (AN)	48	1	49	0.990	0.010	0.005
Jinnan (JN)	56	4	60	0.967	0.033	0.071
Guyuan (GY)	185	7	192	0.982	0.018	0.066

[1]Hardy-Weinberg equilibrium, χ^2 value; $P > 0.05$ in all populations

Derivative-Free Restricted Maximum Likelihood, namely, MTDEREML) was used to analyze each trait with animal models (Boldman et al., 1993). Pedigrees of base population animals were traced back three generations. All analyses were done in two steps, first using a full animal model and then using a reduced animal model. The full animal model included fixed effects of marker genotype, birth year, season of birth (spring vs. fall), age of dam, sire, farm, sex, and random effects of an animal. The reduced model was used in the final analysis. The GLM procedure of software SPSS (Version 13.0) was used to analyze the relationship between the genotypes and traits. The adjusted Linear Model with fixed effects was established:

$$Y_{ijklm} = \mu + S_i + D_{ij} + A_k + G_l + (SG)_{il} + E_{ijklm}$$

where:

Y_{ijklm} = the trait measured on each of the $ijklm^{th}$ animal
μ = the overall population mean
S_i = the fixed effect associated with the i^{th} sire
D_{ij} = the fixed effect associated with j^{th} dam with sire i
A_k = fixed effect due to the k^{th} age
G_l = the fixed effect associated with l^{th} genotype
$(SG)_{il}$ = interaction between the i^{th} sire and the l^{th} genotype
E_{jiklm} = was the random error

An effect associated with farm, sex, and season of birth (spring vs. fall) were not matched in the linear model, as the preliminary statistical analyses indicated that these effects did not significantly influence variability of traits in analyzed populations. The least square means estimates (LSM) with standard errors and multiple range tests for two *POU1F1* genotypes and growth traits were used.

RESULTS

The PCR products of 451 bp including exon 6 and its flanking region were amplified and genotyped by PCR-SSCP. The polymorphic DNA fragments were sequenced and the sequences were deposited in the database (GenBank EF090615 – EF090618). Comparison of the bovine *POU1F1* mRNA sequence NM_174579 and our sequences revealed 2 mutations (G > A and *C* > *T*). The NM_174579:

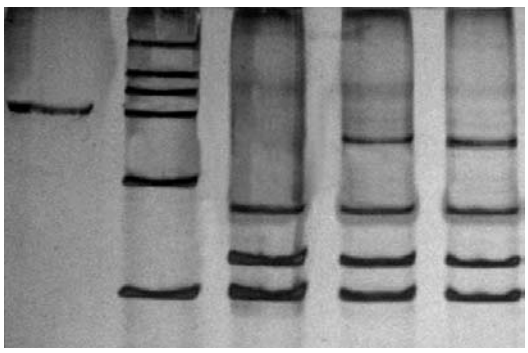

Figure 1. DNA electrophoretic patterns on 10% PAGE after digestion with *Dde*I endonuclease of the PCR fragment containing exon 6 of the bovine *POU1F1* gene Lane 2: Marker DL2000 (2 000 bp, 1 000 bp, 750 bp, 500 bp, 250 bp and 100 bp); Lane 1: non-digested PCR fragment; Lane 3: *CC* genotype; Lanes 4, 5: *CT* genotype

Table 2. Associations of the *Dde*I PCR-RFLP at *POU1F1* locus with weight traits in Nanyang cattle

Traits	*CC* (n = 225) (mean ± SE)	*CT* (n = 26) (mean ± SE)	*P*-value
Birth weight(kg)	29.63 ± 0.12	29.35 ± 0.28	0.644
Body weight of 6 months (kg)	160.33 ± 1.33	158.20 ± 2.27	0.742
Average daily gain of 6 months (g)	0.73 ± 0.01	0.72 ± 0.01	0.663
Body weight of 12 months (kg)	222.10 ± 1.49	216.10 ± 2.17	0.407
Average daily gain of 12 months (g)	0.33 ± 0.01	0.32 ± 0.02	0.920
Body weight of 18 months(kg)	297.27 ± 2.03	297.90 ± 3.32	0.949
Average daily gain of 18 months (g)	0.40 ± 0.02	0.45 ± 0.02	0.618
Body weight of 24 months(kg)	369.59 ± 2.58	353.70 ± 11.90	0.255
Average daily gain of 24 months (g)	0.42 ± 0.02	0.31 ± 0.07	0.364

c.1178G>A mutation was detected in exon 6 and identified a silent allele: Leu (CTG) > Leu (CTA) at the position 276 of 291 AA, which agreed with the Woollard et al. (1994). The other NM_174579: c.1201C>T mutation is described for the first time. It is a missense mutation: Ser (*TCT*) > Phe (TTT) at position 284 (p.S284F), which removed a *Dde*I endonuclease restriction site (CTNAG).

The following fragments were observed after restriction of the 451 bp fragment with *Dde*I: allele *C*: 200 bp, 129 bp, 102 bp and 20 bp; allele *T*: 329 bp, 102 bp and 20 bp. The electrophoresis of the PCR products digested by *Dde*I endonuclease is shown in Figure 1. The 20 bp fragment is too small to be seen on the gel. Frequencies of allele *T* ranged from 0.010 to 0.053 in analyzed populations, all of which were found to be in Hardy-Weinberg equilibrium ($P > 0.05$) (Table 1).

As can be seen from Tables 2 and 3, significant relationships between *Dde*I polymorphism and growth traits in Nanyang cattle (NY, $n = 251$) were not found ($P > 0.05$).

DISCUSSION

In this study, the *Dde*I PCR-RFLP detecting a missense mutation (NM_174579:c.1201C>T) is reported, which results in p.S284F. Serine is a polar amino acid of a small size, while phenylalanine is characterized by aromatic property with big size. So, we presumed that this missense mutation might

Table 3. Associations of the *Dde*I PCR-RFLP at *POU1F1* locus with growth body sizes in Nanyang cattle

Traits	*CC* (n = 225) (mean ± SE)	*CT* (n = 26) (mean ± SE)	*P*-value
Body height of 6 months (cm)	105.95 ± 0.35	106.80 ± 0.78	0.623
Body length of 6 months (cm)	105.60 ± 0.39	104.60 ± 0.92	0.602
Heart girth of 6 months (cm)	129.07 ± 0.46	126.20 ± 1.12	0.206
Height at the hip cross of 6 months (cm)	18.36 ± 0.09	18.10 ± 0.23	0.562
Body height of 12 months (cm)	114.05 ± 0.26	113.60 ± 0.64	0.732
Body length of 12 months (cm)	116.59 ± 0.47	114.40 ± 0.92	0.339
Heart girth of 12 months cm)	141.39 ± 0.50	138.10 ± 1.16	0.187
Height at the hip cross of 12 months (cm)	20.76 ± 0.11	20.35 ± 0.30	0.445
Body height of 18 months (cm)	120.90 ± 0.25	120.90 ± 0.59	0.998
Body length of 18 months (cm)	129.72 ± 0.47	126.10 ± 0.71	0.111
Heart girth of 18 months (cm)	156.12 ± 0.58	155.40 ± 1.62	0.805
Height at the hip cross of 18 months (cm)	23.17 ± 0.12	23.50 ± 0.35	0.586
Body height of 24 months (cm)	126.33 ± 0.31	126.20 ± 0.83	0.932
Body length of 24 months (cm)	138.04 ± 0.49	135.10 ± 1.79	0.246
Heart girth of 24 months (cm)	169.14 ± 0.65	164.90 ± 2.19	0.207
Height at the hip cross of 24 months (cm)	25.26 ± 0.14	25.20 ± 0.48	0.940

change the amino acid property at position 284 of *POU1F1* and affect the encoded protein structure, which could have a direct or indirect effect on bovine production traits. Hence, we analyzed the associations of *Dde*I polymorphism with birth weight, body weight and average daily gain in Nanyang cattle, which are kept in homogenous environments.

Results of statistical evaluation showed no significant relationships between the *Dde*I polymorphism and birth weight, body weight and average daily gain in different growth periods. Apparently, this polymorphism has neither direct nor indirect effects on the genetic variability of the growth traits in the studied population.

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REFERENCES

Boldman K.G., Kriese I.A., Van Vleck L.D., Kachman S.D. (1993): A Manual for Use of MTDFREML. A Set of Programs to Obtain Estimates of Variances and Covariances. ARS, USDA, Washington, USA.

Cohen L.E., Wondisford F.E., Radovick S. (1996): Role of Pit-1 in the gene expression of growth hormone, prolactin, and thyrotropin. Endocrinology and Metabolism Clinics of North America, 25, 523–540.

Li S., Crenshaw E.B., Rawson E.J., Simmons D.M., Swanson L.W., Rosenfeld M.G. (1990): Dwarf locus mutants lacking three pituitary cell-types result from mutations in the POU-domain gene Pit-1. Nature, 347, 528–533.

Mullenbach R., Lagoda P.J., Welter C. (1989): An efficient salt-chloroform extraction of DNA from blood and tissue. Trends in Genetics, 5, 391.

Orita M.H., Iwahana H., Kanazawa H., Sekiya T. (1989): Detection of polymorphism of human DNA by gel electrophoresis as single strand conformation polymorphism. In: Proceedings of the National Academy of Sciences of the United States of America, USA, 86, 2766–2770.

Pfaffle R.W., DiMattia G.E., Parks J., Brown M., Wit J.M., Jansen M., Van der Nat H., Van den Brande J.L., Rosenfel M.G., Ingraham H.A. (1992): Mutation of the POU-specific domain of Pit-1 and hypopituitarism without pituitary hypoplasia. Science, 257, 1118–1121.

Renaville R., Gengler N., Parmentier I., Mortiaux F., Massart S., Bertozzi C., Burny A., Portetelle D. (1997a): Pit-1 gene *HinfI* RFLP and growth traits in double-muscled Belgian Blue Cattle. Journal of Animal Science, 75 (Abstract), 146.

Renaville R., Gengler N., Vrench E., Prandi A., Massart S., Corradini C., Bertozzi C., Mortiaux F., Burny A., Portetelle D. (1997b): Pit-1 gene polymorphism, milk yield, and conformation traits for Italian Holstein-Friesian bulls. Journal of Dairy Science, 80, 3431–3438.

Stančeková K., Vašícčk D., Peškovicová D., Bulla J., Kúbek A. (1999): Effect of genetic variability of the porcine pituitary-specific transcription factor (PIT-1) on carcass traits in pigs. Animal Genetics, 30, 313–315.

Sun H.S., Anderson L.L., Yu T.P., Kim K.S., Klindt J., Tuggle C.K. (2002): Neonatal Meishan pigs show *POU1F1* genotype effects on plasma GH and PRL concentration. Animal Reproduction Science, 69, 223–237.

Woollard J., Schmitz C.B., Freeman A.E., Tuggle C.K. (1994): *Hin*fI polymorphism at the bovine *PIT1* locus. Journal of Animal Science, 72, 3267.

Woollard J., Tuggle C.K., Ponce de Leon F.A. (2000): Rapid communication: Localization of *POU1F1* to bovine, ovine, and caprine 1q21-22. Journal of Animal Science, 78, 242–243.

Xue K., Chen H., Wang S., Cai X., Liu B., Zhang C.F., Lei C.Z., Wang X.Z., Wang Y.M., Niu H. (2006): Effect of genetic variations of the *POU1F1* gene on growth traits of Nanyang cattle. Acta Genetica Sinica, 33, 901–907.

Yu T.P., Tuggle C.K., Schmitz C.B., Rothschild M.F. (1995): Association of PIT1 polymorphisms with growth and carcass traits in pigs. Journal of Animal Science, 73, 1282–1288.

Zhao Q., Davis M.E., Hines H.C. (2004): Associations of polymorphisms in the Pit-1 gene with growth and carcass traits in Angus beef cattle. Journal of Animal Science, 82, 2229–2233.

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