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Genetic variation, association analysis, and expression pattern of *SMAD3* gene in Chinese cattle

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ABSTRACT: SMAD3, a member of SMAD transcription factors, plays a key role in transforming growth factor-beta (TGF-β) signalling pathway and regulation of muscle growth. However, there was no strong evidence of association between *SMAD3* polymorphisms and body traits in animals. In this study, single nucleotide polymorphisms (SNPs) in *SMAD3* gene were detected in four Chinese cattle breeds (Qinchuan, Jiaxian, Nanyang, and Caoyuan) by using DNA pool sequencing and PCR-RFLP, and their effects on gene expression and growth traits were evaluated in Qinchuan cattle. The results showed that four novel SNPs (NC_007308.5 c.-2017A>G, g.101664C>G, g.105829A>G, and g.114523A>G) in promoter, intron 3, and intron 5 were found in four cattle breeds. NC_007308.5 c.-2017A>G and g.114523A>G were significantly ($P < 0.05$) associated with *SMAD3* gene expression. Furthermore, the four SNPs were strikingly ($P < 0.05$) associated with rump length, chest girth, and body weight in two-year-old Qinchuan cattle. Our results provided the evidence that SNPs in *SMAD3* were associated with cattle traits, which showed the possibility that the four SNPs could be novel molecular markers for beef cattle breeding and genetics.

Keywords: *SMAD3* polymorphisms; mRNA level; body traits; bovine

INTRODUCTION

SMADs (Sma and Mad-related proteins) are a class of intracellular proteins that transmit extracellular signals from TGF-β superfamily members (Derynck and Zhang 2003; Shi and Massague 2003). TGF-β ligand binds to the subsequent receptor complex and results in C-terminal phosphorylation of SMAD2 and SMAD3. Receptor-activated SMADs form a complex, with the common mediator SMAD4 involved. The SMADs complexes then translocate into the nucleus to control target genes' expression. Disruption of the SMAD+ TGF-β signalling pathway is causal to the pathogenesis of several disorders and diseases (Fiocchi 2001; Bonniaud et al. 2005).

SMAD3 could strictly inhibit the muscle development. First, it was known that myogenic regulatory factors (MRFs) and myocyte enhancer factor 2 (MEF2) families were two main groups which regulate myocyte formation and differentiation (Perry and Rudnicki 2000). And the intracellular effector SMAD3 could inhibit myogenesis by repressing the activity of the MyoD family through interfering with the assembly of the bHLH transcription factor on E-box sequences (Liu et al. 2001). Also, TGF-β activated SMAD3 was shown to repress MEF2-dependent transcription and inhibit terminal

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differentiation in myogenesis (Liu et al. 2004). Second, myostatin, a member of TGF-β superfamily, can negatively regulate muscle mass (McPherron and Lee 2002), and was shown to induce skeletal muscle wasting through SMAD3 mediated expression of functional genes (Lokireddy et al. 2011). In addition, SMAD3 deficiency resulted into impaired muscle regeneration (Ge et al. 2011). Therefore, it is hypothesized that the mutations in *SMAD3* gene may be relevant to cattle muscle growth and contribute to phenotypic traits.

Up to date, the polymorphisms of *SMAD3* gene and their effects on livestock animals have not been reported. In this study, it was aimed to explore *SMAD3* SNPs in Chinese cattle and examine their potential effects on gene expression and growth traits. It provided an insight into the utilization of the *SMAD3* polymorphisms as markers in beef cattle breeding program.

MATERIAL AND METHODS

Genomic DNA obtaining and data collecting. All experiments procedures were approved by the Review Committee for the Use of Animal Subjects of Northwest A&F University, and animal experimentation, including fetal and adult sample collection, was followed by the agreement of ethical commission. A total of 999 cattle were collected in four Chinese breeds: Qinchuan cattle in Shaanxi province (QC, n = 519), Jiaxian cattle in Henan province (JX, n = 120), Nanyang cattle in Henan province (NY, n = 220), Chinese Caoyuan cattle in Jilin province (CY, n = 140). All test animals of each breed were cows reared at one farm. They were in good physical condition and had similar conditions of feeding and management in accordance with mandatory standards. Calves were weaned at a mean age of 6 months, and had been raised from weaning to slaughter on a corn-corn silage diet. Additionally, animals from each breed were unrelated for at least three generations, with the aim of having diverse lineages within each breed. Genomic DNA was extracted from blood samples, diluted to a standard concentration (50 ng/μl), and stored at –80°C for the subsequent use (Sambrook and Russell 2001). Growth traits of two-year-old QC cattle, including body height, height at hip cross, body length, chest girth, chest width, chest depth, rump length, hucklebone width, hip width, and body weight, were collected for association analysis. They were collected according to the measurement of Gilbert et al. 1993.

Primer design and PCR amplification. A total of ten PCR primer pairs (Table S1) were designed for the coding region and promoter region (–2000 bp to –1 bp away from the translation start site) of bovine *SMAD3* gene based on the reference sequence (GenBank accession No. NC_007308.5). PCR was performed in 25 μl of reaction volume, containing 50 ng/μl genomic DNA, 10μM of each primer, 1× buffer (including 1.5mM $MgCl_2$), 200μM dNTPs, and 0.6 U of *Taq* DNA polymerase (MBI, Vilnius, Lithuania). 50 individual genomic DNA samples randomly chosen from each cattle breed were pooled as PCR template (Norton et al. 2004). The PCR reaction process, initial denaturation at 95°C for 5 min, 35 cycles at 94°C for 30 s, annealing at selected temperatures for 30 s, extension times at 72°C depended on the amplification product segment (Table S1) with a final extension at 72°C for 10 min, was carried out in a PCR thermal cycler system (Bio-Rad Laboratories, Hercules, USA).

SNPs exploring and genotyping. PCR products were sequenced in a bidirectional way by Genscript Company (Nanjing, China). Sequence alignment of the sequenced reads against the reference sequence (GenBank accession No. NC_007308.5) was performed using BioXM software (Version 2.6, 2010) to preliminarily search mutations. After that, forced polymerase chain reaction-restriction fragment length polymorphisms (forced PCR-RFLP) were employed to detect SNPs, and the primers are shown in Table S1. 7 μl PCR products were digested with 2 U *Xba*I, *Msp*I, *Pst*1, and *Sal*I (10 U/μl; TaKaRa, Dalian, China), respectively, for four detected SNPs in *SMAD3* gene at 37°C for 10 h, and then the PCR-RFLP products were checked by electrophoresis on a 1.5–3% agarose gel stained with ethidium bromide.

Tissue collection, RNA isolation, and qPCR. Heart, liver, spleen, lung, kidney, small intestines, *longissimus dorsi* muscle and adipose (fat) were obtained from three QC fetal individuals (fetus: 90 days) and three QC adults (24 months old) for total RNA isolation. And muscle tissues were gathered from adult cattle (n = 26) for both total RNA and genomic DNA extraction. No animals exhibited adverse health conditions. After collection, tissues were immediately frozen in liquid nitrogen and stored at –80°C. RNA was extracted using Trizol reagent (TaKaRa) according to the

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manufacturer's protocol. RNA purity and concentration were checked by NanoDrop ND-1000 spectrophotometer (Nanodrop, Wilmington, USA), and then stored at –80°C. After RNA quality was confirmed on 0.8% agarose gel electrophoresis, cDNA synthesis was done by using a PrimeScript RT Reagent Kit (TaKaRa) in 20 µl PCR volume with 2 µg RNA. The reaction was carried out in PCR Thermal Cycler Dice system, at 42°C for 2 min, followed by 37°C for 15 min and 85°C for 5 min. cDNA was stored at –20°C for subsequent use.

SYBR Green quantitative PCR was performed on a Bio-Rad CFX 96 Real-Time PCR Detection System, and the primers in qPCR are shown in Table S2. *GAPDH* was used as an endogenous reference gene as described by Xu et al. (2013). A total 12 µl reaction system contained 6 µl SYBR Premix Ex Taq^{TM} II (TaKaRa). The thermal profile was 95°C for 30 s, followed by 39 cycles of 95°C for 5 s, 60°C for 30 s, and 65°C for 5 s. The relative mRNA expression level of target gene was calculated based on the level of *GAPDH* cDNA by using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis. Genotypic and allelic frequencies of four SNPs were calculated. Genetic parameters were calculated in POPGENE software (Version 1.32, 2010), including heterozygosity (He), homozygosity (Ho), effective allele numbers (Ne), and PIC (polymorphism information content), according to Nei's methods (Nei and Roychoudhury 1974). Hardy-Weinberg equilibrium was tested based on chi-square test for different mutation loci, $P < 0.05$ was considered statistically significant (Yeh et al. 1999). SHEsis online software (http://analysis2.bio-x.cn/myAnalysis.php) was utilized to analyze linkage disequilibrium (Shi and He 2005).

The association of the genotypes of *SMAD3* gene with growth trait was calculated using the General Linear Model (GLM) procedure in SPSS software (Version 18.0). The effect of genotype was included in this model. The reduced linear model was:

$$Y_{ij} = \mu + G_i + \varepsilon_{ij}$$

where:

Y_{ij} = observation value of the target traits

μ = overall mean of each target trait

G_i = fixed effect of j^{th} genotype of each SNP loci

ε_{ij} = residual error

The effects associated with farm, sex, sire, age, and season of birth (spring vs autumn) were not matched in the linear model, as the preliminary statistical analysis indicated that they did not have a significant influence on the traits (Zhang et al. 2014).

RESULTS

Identification of sequence variants. In this study, four novel SNPs, c.-2017A>G (SNP1), g.101664C>G (SNP2), g.105829A>G (SNP3), and g.114523A>G (SNP4) were revealed (Figure S1) by sequencing DNA pool and alignment against the GenBank reference sequence (NC_007308.5; transcription start site of *SMAD3*: 13717417; transcription end site: 13839788; translation start site: 13717719). SNP1 was found in the promoter region, and *Xba*I restriction endonuclease was used to detect this locus in PCR-RFLP. SNP2 in intron 3, SNP3 in intron 5, and SNP4 in intron 5 were genotyped by using *Msp*I, *Pst*1, and *Sal*I restriction endonuclease, respectively. Genotyping electrophoregram of the four SNPs is shown in Figure S2.

Genetic diversity and linkage disequilibrium analysis. Genotypic frequencies, allelic frequencies, and other genetic parameters of four SNPs in the four cattle populations are shown in Table 1. PIC values showed that QC breed at SNP1 and SNP3, NY breed at SNP3 and SNP4, JX breed only at SNP3, and CY breed at SNP1, SNP2, and SNP3 possessed middle genetic diversity ($0.250 < PIC < 0.500$). The χ^2 test indicated that SNP1, SNP2, and SNP3 in both JX and NY, SNP2 in CY, and SNP4 in JX were in accordance with Hardy-Weinberg equilibrium ($P > 0.05$), and other SNPs in four cattle breeds were severely out of the Hardy-Weinberg equilibrium ($P < 0.05$).

Linkage disequilibrium (LD) pairwise analysis results for the SNPs in four cattle breeds are shown in Table 2. SNP2 and SNP4 in JX and NY as well as SNP3 and SNP4 in QC were in perfect linkage equilibrium (LE, $r^2 = 0$), however, other pairwise loci were in weak linkage disequilibrium ($r^2 < 0.33$) (Ardlie et al. 2002).

Expression patterns of SMAD3 gene. The expression patterns of *SMAD3* gene in various tissues (skeletal muscle, heart, liver, lung, brain, spleen, kidney, and small intestine) from three QC fetal and three adult cattle are shown in Figure 1. *SMAD3* gene showed a wide range of expression profile in two development stages of QC cattle. At fetal stage, *SMAD3* expression was high in kidney, medium in spleen and intestines, and

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Table 1. Genetic diversity analysis of *SMAD3* SNPs loci in four Chinese cattle breeds

Loci	Breed[1]	Genotypic frequency			Allelic frequency		Ho	He	Ne	PIC	*P* (HWE)
		AA	*AG*	*GG*	*A*	*G*					
SNP1	QC	0.025	0.399	0.575	0.225	0.775	0.651	0.349	1.536	0.288	< 0.05
	JX	0.000	0.101	0.899	0.050	0.950	0.904	0.096	1.106	0.091	> 0.05
	NY	0.000	0.111	0.889	0.056	0.944	0.895	0.105	1.117	0.099	> 0.05
	CY	0.029	0.664	0.307	0.361	0.639	0.539	0.461	1.856	0.355	< 0.05
		CC	*CG*	*GG*	*C*	*G*					
SNP2	QC	0.052	0.151	0.797	0.127	0.873	0.778	0.222	1.286	0.198	< 0.05
	JX	0.000	0.026	0.974	0.013	0.987	0.974	0.026	1.026	0.025	> 0.05
	NY	0.016	0.297	0.688	0.164	0.836	0.726	0.274	1.378	0.237	> 0.05
	CY	0.014	0.171	0.814	0.100	0.900	0.820	0.180	1.220	0.164	> 0.05
		AA	*AG*	*GG*	*A*	*G*					
SNP3	QC	0.378	0.427	0.194	0.592	0.408	0.517	0.483	1.934	0.366	< 0.05
	JX	0.268	0.464	0.268	0.500	0.500	0.500	0.500	2.000	0.375	> 0.05
	NY	0.302	0.476	0.222	0.540	0.460	0.503	0.497	1.987	0.373	> 0.05
	CY	0.571	0.407	0.021	0.775	0.225	0.651	0.349	1.536	0.288	< 0.05
		AA	*AG*	*GG*	*A*	*G*					
SNP4	QC	0.825	0.175	0.000	0.912	0.088	0.840	0.160	1.190	0.147	< 0.05
	JX	0.863	0.137	0.000	0.932	0.068	0.873	0.127	1.146	0.119	> 0.05
	NY	0.462	0.492	0.046	0.708	0.292	0.586	0.414	1.706	0.328	< 0.05
	CY	0.179	0.821	0.000	0.589	0.411	0.516	0.484	1.938	0.367	< 0.05

Ho = observed heterozygosity, He = expected heterozygosity, Ne = effective allele numbers, PIC = polymorphism information content, *P*(HWE) = Hardy-Weinberg equilibrium *P*-value

[1]breeds: QC = Qinchuan (*n* = 519), JX = Jiaxian (*n* = 120), NY = Nanyang (*n* = 220), CY = Chinese Caoyuan (*n* = 140)

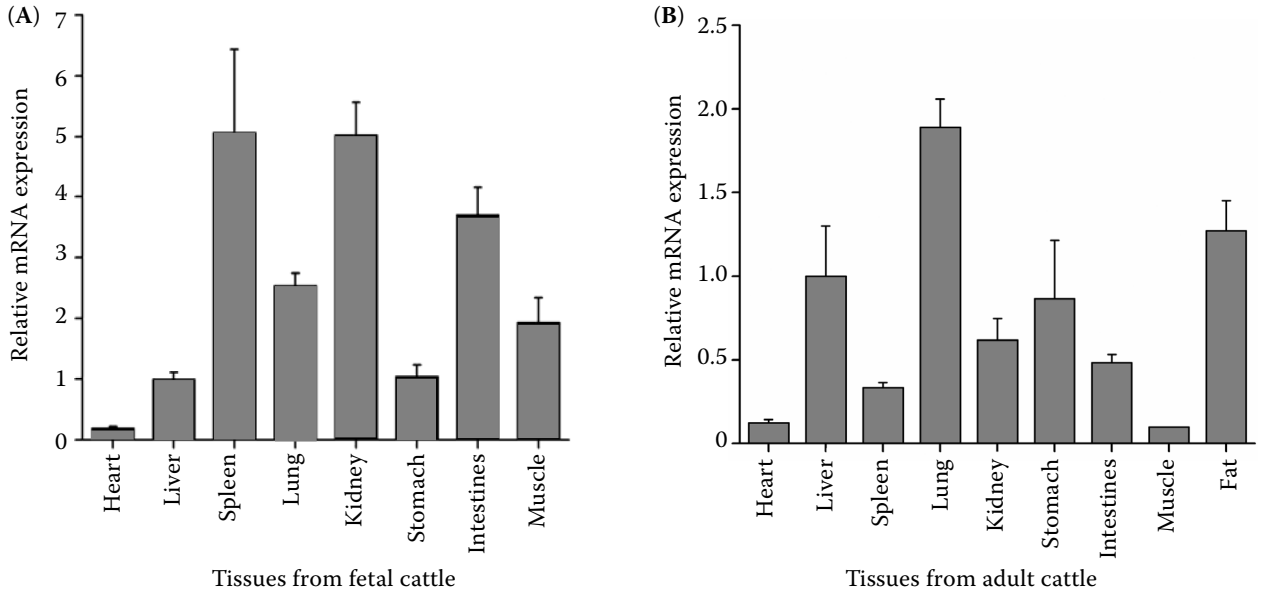

Figure 1. *SMAD3* gene expression patterns of tissues in Qichuan cattle

(**A**) relative mRNA level of *SMAD3* gene in fetal tissues, (**B**) relative mRNA level of *SMAD3* gene in adult tissues
expression level was calculated with $2^{-\Delta\Delta Ct}$ value and normalized to *GAPDH* gene
error bars represent standard deviations of three different biological replicates

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Table 2. Linkage disequilibrium analysis between four SNPs within *SMAD3* gene in four cattle populations

SNPs[1]	QC		JX		NY		CY	
	r^2	D'	r^2	D'	r^2	D'	r^2	D'
SNP1-2	0.002	0.065	0.002	0.999	0.001	0.301	0.033	0.726
SNP1-3	0.001	0.039	0.049	0.619	0.005	0.364	0.042	0.504
SNP1-4	0.002	0.287	0.008	0.112	0.003	0.360	0.254	0.804
SNP2-3	0.021	0.323	0.046	1.000	0.003	0.163	0.268	0.837
SNP2-4	0.010	0.831	0.000	0.252	0.000	0.025	0.007	0.213
SNP3-4	0.000	0.031	0.015	0.282	0.009	0.117	0.053	0.358

QC = Qichuan, JX = Jiaxian, NY = Nanyang, CY = Chinese Caoyuan

[1]SNPs: estimated values of linkage disequilibrium (r^2 and D') between polymorphism pairs

low in heart (Figure 1A). At adult stage, the relative mRNA level of *SMAD3* was the highest in lung tissue, followed by fat and liver tissue (Figure 1B).

Transcript levels of different genotypes in four SNPs. To study biological roles of the four SNPs, mRNA expression levels of *SMAD3* gene were compared in different genotypes in adult QC muscle tissue. qPCR analysis showed that the SNPs were significantly associated with gene expression (Figure 2), where individuals with *GG* at SNP1 had significantly higher mRNA expression level compared with *AG* type ($P < 0.05$), and similarly, *AG* genotype at SNP4 also presented obviously higher transcript activity than *AA* ($P < 0.01$). No strong significance was observed in SNP2 and SNP3, but it was noted that *CC* at SNP2 and *GG*

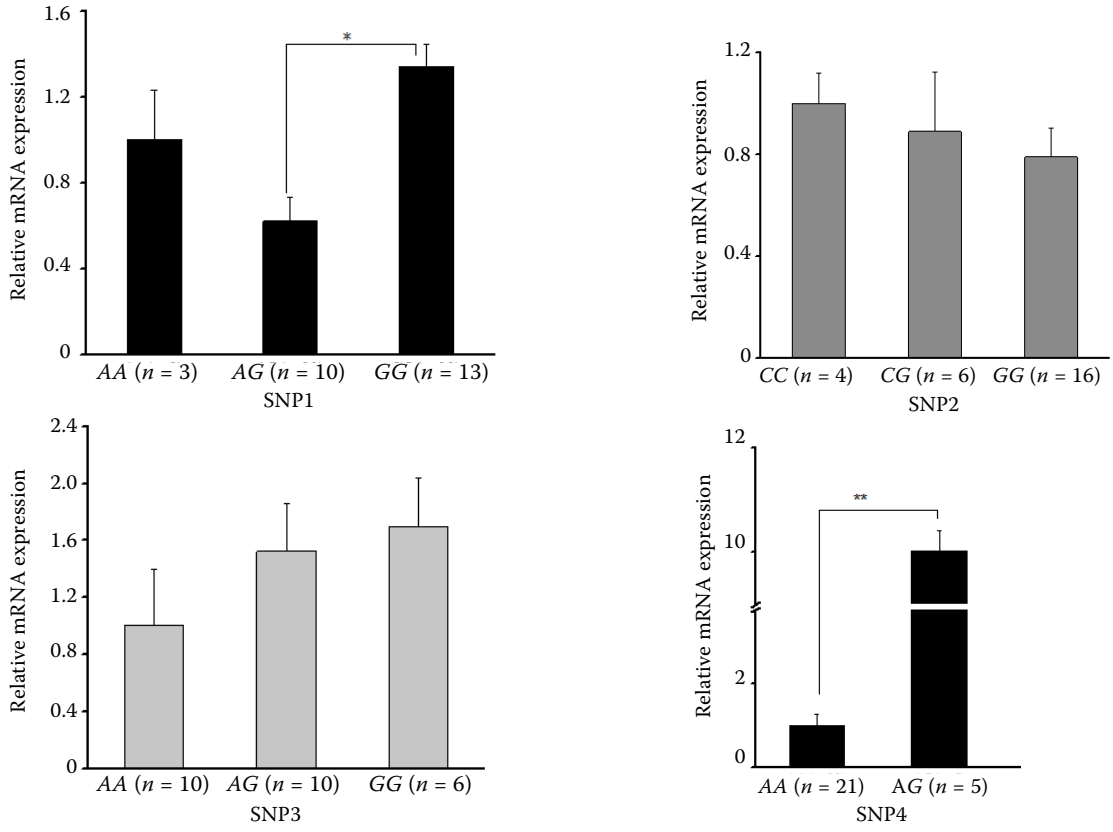

Figure 2. Comparison of *SMAD3* gene relative expression values of different genotypes at four SNPs in adult Qichuan cattle

bars represent standard error

*$P < 0.05$, **$P < 0.01$

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Table 3. Associations of four SNPs of *SMAD3* gene with growth traits in Qinchuan cattle

Loci	Genetype	*n*	Growth traits (mean ± SE)				
			CG (cm)	CW (cm)	RL (cm)	HW (cm)	BW (kg)
SNP1	*AA*	10	172.25 ± 5.78	38.13 ± 1.74	41.50 ± 0.87^{ab}	21.50 ± 1.89	382.25 ± 27.32
	AG	71	173.44 ± 1.25	36.74 ± 0.51	44.25 ± 0.35^{a}	22.02 ± 0.43	379.37 ± 7.67
	GG	97	174.05 ± 0.98	36.51 ± 0.39	43.14 ± 0.32^{b}	22.14 ± 0.35	380.25 ± 6.94
P-value			0.887	0.716	0.032	0.926	0.994
SNP2	*CC*	10	169.80 ± 5.39	33.90 ± 1.52^{ab}	45.40 ± 1.36	21.60 ± 0.93	370.20 ± 27.12
	CG	27	173.50 ± 1.79	35.18 ± 0.83^{b}	43.45 ± 0.81	21.34 ± 0.62	380.23 ± 11.79
	GG	141	173.94 ± 0.84	36.95 ± 0.33^{a}	43.50 ± 0.25	22.20 ± 0.30	380.23 ± 5.68
P-value			0.661	0.049	0.413	0.545	0.948
SNP3	*AA*	67	175.13 ± 1.41	37.54 ± 0.49^{a}	43.62 ± 0.40	22.75 ± 0.43	388.91 ± 9.71
	AG	76	173.13 ± 1.09	36.39 ± 0.47^{ab}	43.59 ± 0.34	21.95 ± 0.45	374.45 ± 7.12
	GG	35	172.57 ± 1.42	35.47 ± 0.64^{b}	43.31 ± 0.60	21.10 ± 0.42	374.83 ± 8.97
P-value			0.369	0.039	0.889	0.077	0.393
SNP4	*AA*	145	174.62 ± 0.82^{a}	36.75 ± 0.34	43.75 ± 0.26	22.15 ± 0.30	385.21 ± 5.44^{a}
	AG	33	169.62 ± 1.86^{b}	36.10 ± 0.71	42.53 ± 0.59	21.75 ± 0.59	354.33 ± 12.69^{b}
P-value			0.013	0.425	0.054	0.579	0.021

CG = chest girth, CW = chest width, RL = rump length, HW = hucklebone width, BW = body weight of cattle aged two years
^{a,b}values differ significantly at $P < 0.05$

at SNP3 showed higher relative mRNA expression level than the other two genotypes.

Association analysis of different genotypes and growth traits in QC breed. Associations of these four SNPs with growth traits were analyzed in QC cattle and the results are shown in Table 3. SNP1 was associated with rump length of two-year-old QC cattle ($P < 0.05$). Additionally, a significant increase in chest width was observed in *GG* at SNP2 ($P < 0.05$). At SNP3, genotype *AA* had significantly greater chest width than *AG* and *GG* genotypes. SNP4 was also significantly associated with chest girth and body height where the values of *AA* genotype were significantly higher than those of *AG* genotype ($P < 0.05$).

DISCUSSION

SMAD3 was a key factor to transmit TGF-β signals from cell-surface to nuclei and involved in multiple cellular activities, including cell proliferation, differentiation, and apoptosis (Derynck and Zhang 2003). It was shown that SMAD3 inhibited muscle development by repressing the activities of MRFs and MEF2 factors (Liu et al. 2001, 2004). Therefore, we hypothesized that the sequence polymorphisms within *SMAD3* gene would lead to phenotypic differences in cattle. Indeed, our results indicated that the detected four SNPs were associated with *SMAD3* gene expression and growth traits in Chinese cattle significantly.

Genetic characteristics of the four SNPs in *SMAD3* gene were evaluated in Chinese cattle breeds. Interestingly, *GG* genotype at SNP4 was not found in QC, JX, and CY breeds. These results indicated that the allele *G* may be a harmful mutation leading to animal growth deficiency, and thus disappeared due to natural selection (Huang et al. 2010; He et al. 2014). However, further researches are needed to explain the mechanism of the missing genotypes observed in this study.

Additionally, *SMAD3* mRNA presence in several bovine tissues at different growth stages was investigated and the results suggested that this gene was expressed ubiquitously. The presence of *SMAD3* transcript in embryonic period as well as adult period may reveal the evidence of multiple roles of this gene in diverse organs and tissues (Olson 1992; Choy et al. 2000).

Strong associations ($P < 0.05$) were observed between four SNPs and growth traits of two-year-old QC cattle, including rump length, chest girth, and body weight. Numerous researches revealed that mutations in 5'-UTR and introns could influence

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transcription and thus alter biological function of genes as well as phenotype (Greenwood and Kelsoe 2003; Van Laere et al. 2003). In this study, *AG* genotype at SNP1 and *AA* genotype at SNP4 showed lower transcription levels, whereas performed better rump length, chest girth, and body weight. This may be partly due to the repression effects of SMAD3 on myogenesis. SMAD3 was essential for regulating muscle growth by interacting with other muscle specific regulatory factors (Liu et al. 2001; Ge et al. 2011; Sriram et al. 2014). It was known that muscle development and growth could partly determine body weight in animals (Huang et al. 2015). Thus, the current observations in this study together with previous studies raised the possibility that SMAD3 was a potential mediator of bovine body weight and phenotype.

In conclusion, four novel SNPs in the bovine *SMAD3* were identified in four Chinese cattle breeds. Substantial differences were presented in genetic parameters of SNPs in these cattle breeds. Moreover, association analysis showed the four SNPs were both significantly associated with gene expression and growth traits in Chinese cattle. However, further researches and validation of the various allelic effects, molecular mechanisms, and the bioactivity are needed to evaluate the potential of *SMAD3* polymorphisms as molecular markers in cattle breeding and genetics.

REFERENCES

Ardlie K.G., Kruglyak L., Seielstad M. (2002): Patterns of linkage disequilibrium in the human genome. Nature Reviews Genetics, 3, 299–309.

Bonniaud P., Margetts P.J., Ask K., Flanders K., Gauldie J., Kolb M. (2005): TGF- and Smad3 signaling link inflammation to chronic fibrogenesis. Journal of Immunology, 175, 5390–5395.

Choy L., Skillington J., Derynck R. (2000): Roles of autocrine TGF-β receptor and Smad signaling in adipocyte differentiation. The Journal of Cell Biology, 149, 667–681.

Derynck R., Zhang Y.E. (2003): Smad-dependent and Smad-independent pathways in TGF-β family signalling. Nature, 425, 577–584.

Fiocchi C. (2001): TGF-β/Smad signaling defects in inflammatory bowel disease: mechanisms and possible novel therapies for chronic inflammation. Journal of Clinical Investigation, 108, 523–526.

Ge X., McFarlane C., Vajjala A., Lokireddy S., Ng Z.H., Tan C.K., Wahli W., Sharma M., Kambadur R. (2011): Smad3 signaling is required for satellite cell function and myogenic differentiation of myoblasts. Cell Research, 21, 1591–1604.

Gilbert R.P., Bailey D.R.C., Shannon N.H. (1993): Linear body measurements of cattle before and after 20 years of selection for postweaning gain when fed two different diets. Journal of Animal Science, 71, 1712–1720.

Greenwood T.A., Kelsoe J.R. (2003): Promoter and intronic variants affect the transcriptional regulation of the human dopamine transporter gene. Genomics, 82, 511–520.

He H., Zhang H.L., Li Z.X., Liu Y., Liu X.L. (2014): Expression, SNV identification, linkage disequilibrium, and combined genotype association analysis of the muscle-specific gene CSRP3 in Chinese cattle. Gene, 535, 17–23.

Huang Y.Z., Zhang E.P., Chen H., Wang J., Li Z.J., Huai Y.T., Ma L., Lan X.Y., Ren G., Lei C.Z., Fang X.T., Wang J.Q. (2010): Novel 12-bp deletion in the coding region of the bovine NPM1 gene affects growth traits. Journal of Applied Genetics, 51, 199–202.

Huang S., Yang S., Guo J., Yan S., Gaertig M.A., Li S., Li X.J. (2015): Large polyglutamine repeats cause muscle degeneration in SCA17 mice. Cell Reports, 13, 196–208.

Liu D., Black B.L., Derynck R. (2001): TGF-β inhibits muscle differentiation through functional repression of myogenic transcription factors by Smad3. Genes and Development, 15, 2950–2966.

Liu D., Kang J.S., Derynck R. (2004): TGF-β-activated Smad3 represses MEF2-dependent transcription in myogenic differentiation. The EMBO Journal, 23, 1557–1566.

Lokireddy S., McFarlane C., Ge X., Zhang H., Sze S.K., Sharma M., Kambadur R. (2011): Myostatin induces degradation of sarcomeric proteins through a Smad3 signaling mechanism during skeletal muscle wasting. Molecular Endocrinology, 25, 1936–1949.

McPherron A.C., Lee S.J. (2002): Suppression of body fat accumulation in myostatin-deficient mice. The Journal of Clinical Investigation, 109, 595–601.

Nei M., Roychoudhury A. (1974): Sampling variances of heterozygosity and genetic distance. Genetics, 76, 379–390.

Norton N., Williams N.M., O'Donovan M.C., Owen M. (2004): DNA pooling as a tool for large-scale association studies in complex traits. Annals of Medicine, 36, 146–152.

Olson E.N. (1992): Interplay between proliferation and differentiation within the myogenic lineage. Developmental Biology, 154, 261–272.

Perry R.L., Rudnicki M.A. (2000): Molecular mechanisms regulating myogenic determination and differentiation. Frontiers in Bioscience, 5, 750–767.

Sambrook J., Russell D.W. (2001): Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York, USA.

doi: 10.17221/34/2015-CJAS

Shi Y.G., Massague J. (2003): Mechanisms of TGF-β signaling from cell membrane to the nucleus. Cell, 113, 685–700.

Shi Y.Y., He L. (2005): SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Research, 15, 97–98.

Sriram S., Subramanian S., Juvvuna P.K., Ge X., Lokireddy S., McFarlane C.D., Wahli W., Kambadur R., Sharma M. (2014): Myostatin augments muscle-specific ring finger protein-1 expression through an NF-kB independent mechanism in SMAD3 null muscle. Molecular Endocrinology, 28, 317–330.

Van Laere A.S, Nguyen M., Braunschweig M., Nezer C., Collette C., Moreau L., Archibald A.L., Haley C.S., Buys N., Tally M., Andersson G., Georges M., Andersson L. (2003): A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. Nature, 425, 832–836.

Xu Y., Zhang L.Z, Shi T., Zhou Y., Cai H.F, Lan X.Y., Zhang C.L., Lei C.Z., Chen H. (2013): Copy number variations of MICAL-L2 shaping gene expression contribute to different phenotypes of cattle. Mammalian Genome, 24, 508–516.

Yeh F.C., Yang R., Boyle T. (1999): POPGENE Version 3.1, Microsoft Window-based Freeware for Population Genetic Analysis. University of Alberta, Edmonton, Canada.

Zhang L., Yang M., Li C., Xu Y., Sun J., Lei C., Lan X., Zhang C., Chen H. (2014): Identification and genetic effect of a variable duplication in the promoter region of the cattle ADIPOQ gene. Animal Genetics, 45, 171–179.

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