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Identification of single nucleotide polymorphisms of the signal transducer and activator of transcription 3 gene (*STAT3*) associated with body measurement and carcass quality traits in beef cattle

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ABSTRACT. Previous studies have shown that the signal transducer and activator of transcription 3 gene (*STAT3*) is involved in lipid storage and energy metabolism, suggesting that *STAT3* is a potential candidate gene that affects body measurement and carcass quality traits in animals. Therefore, the aim of this study was to identify polymorphisms in bovine *STAT3* and to analyze their possible associations with body measurement and carcass quality traits in 493 individuals of 2 native Chinese cattle breeds: Qinchuan (N = 371) and Jiaxian cattle (N = 122). DNA sequencing and polymerase chain reaction-restriction fragment

length polymorphism (PCR-RFLP) were employed to detect *STAT3* single nucleotide polymorphisms (SNPs). We found 5 SNPs: 1 in an exon (g.65812G>A: exon 16) and 4 in introns (g.43591G>A: 13 intron, g.67492T>G: 19 intron, g.67519T>C: 19 intron, and g.68964G>A: 20 intron). Both g.65812G>A and g.68964G>A were not in Hardy-Weinberg equilibrium (HWE), whereas individual frequencies of each genotype were consistent with HWE for other SNPs in Qinchuan cattle populations. For the Jiaxian cattle, the genotype distributions of the 4 mutations were in HWE except for g.67519T>C. The results indicate that these SNPs have a significant association with some body measurements and carcass quality traits ($P < 0.05$ or $P < 0.01$). Therefore, *STAT3* might have potential effects on production traits in beef cattle populations and could be used for marker-assisted selection.

Key words: STAT3; SNP; Body measurement traits; Carcass quality traits

INTRODUCTION

Signal transducer and activator of transcription (STAT) belongs to a family of latent cytoplasmic transcription factors that convey signals from the cell membrane to the nucleus (Schust et al., 2006). They control key cellular and physiological processes, and have roles in immune regulation, apoptosis, lipid metabolism, and colorectal cancer (Dinasarapu et al., 2013). Seven STAT family members (STAT1, 2, 3, 4, 5a, 5b, and 6) have been identified in mammals (Lidija, 2013). Each of them is activated by specific extracellular ligands, including cytokines, growth factors, and hormones (Darnell, 1997; Levy and Darnell, 2002). Janus kinases (JAKs) have tyrosine kinase activity and can bind certain cell-surface cytokines (Liu et al., 2012a). Binding of ligands to receptors leads to the activation of JAKs, which can directly phosphorylate STATs and subsequently activate the transcription of target genes (Yang and George, 2008). STAT3, a transcription factor expressed in proliferating pre-adipocytes and adipocytes (Deng et al., 2000), is activated through phosphorylation of a single tyrosine (T705) in response to stimulation with cytokines, growth factors, or nutrients (Turkson and Jove, 2000). Phosphorylated STAT3 undergoes homo-dimerization and translocates to the nucleus, where it can bind to DNA and regulate the transcription of specific genes (Sen et al., 2009), such as PPARγ, PRDM16, and C/EBPβ. Zhang et al. (2011) demonstrated that the JAK2 inhibitor AG490 and siRNA could partially inhibit STAT3 activation and inhibit differentiation of 3T3-L1 adipocytes (Zhang et al., 2011). In these processes, STAT3 interacts with C/EBPβ by binding the distal region of the C/EBPβ promoter at the early stage of adipogenesis. Treatment of 3T3-L1 pre-adipocytes with troglitazone (a synthetic PPARγ agonist) abolished STAT3-inhibitor and RNAi-mediated suppression of adipogenesis, suggesting that STAT3 regulates adipocyte differentiation via PPARγ (Wang et al., 2010). Mice lacking the JAK tyrosine kinase member Tyk2 became progressively obese due to aberrant development of Myf5+ brown adipose tissue (BAT). However, the constitutively active form of *STAT3* could enhance stability of PRDM16 protein, leading to improved BAT development, normal levels of insulin, and significantly lower body weights in Tyk2-/- mice (Derecka et al., 2012). Studies using Ras-transformed mouse embryonic fibroblasts showed that ATP production was restricted in the absence of *STAT3*, suggesting that mitochondrial STAT3 is closely associated with cellular

ATP levels (Gough et al., 2009). Taken together, these findings suggest that *STAT3* might be a potential candidate gene for the selection of growth-related traits in livestock.

Since there are no reports of associations between *STAT3* variants and body measurement and carcass quality traits in herbivore breeding, the current study was designed to detect the genetic variation of *STAT3* in 493 Chinese cattle, and to explore the possible genetic association between variants of *STAT3* and body measurement and carcass quality traits, which will benefit cattle breeding and genetics.

MATERIAL AND METHODS

Animals, DNA samples, and data collection

Blood samples were obtained from 493 individuals (unrelated for at least 3 generations) of 2 native Chinese cattle breeds: Qinchuan (371) and Jiaxian cattle (122). In this study, the Qinchuan animals were from the National Beef Cattle Improvement Center's experimental farm (Yangling Shaanxi, China), and the Jiaxian animals were from the Jiaxian Cattle breeding farm (Jiaxian County, Henan Province, China).

Genomic DNA of 493 cattle was isolated from blood samples treated with 2% heparin, according to the standard phenol chloroform protocol, and stored at -80°C. DNA content was estimated spectrophotometrically, and then the DNA was diluted to 50 ng/μL. All the DNA samples were stored at -20°C for subsequent analysis.

We quantified the body measurement (body length, withers height, and chest circumference) and carcass quality (backfat thickness, ultrasound loin muscle area, and intramuscular fat content) traits of 371 Qinchuan cattle.

Genotyping

Primers used to amplify bovine *STAT3* were designed from a published gene sequence (GenBank accession No. XM_010816228.1; Table 1). PCR amplification was performed in a 20-μL volume mixture containing 50 ng DNA, 10 pM of each primer, 1X buffer (including 1.5 mM $MgCl_2$), 200 uM dNTPs, and 0.5 U Taq DNA polymerase (TaKaRa, Shiga, Japan). PCR conditions were as follows: 5 min at 94°C for 5 min, 35 cycles at 94°C for 30 s, annealing for 30 s, 72°C for 35 s, and a final extension of 10 min at 72°C.

Single nucleotide polymorphisms (SNPs) were identified through DNA sequencing of different regions. A DNA pool (30 ng/μL per cow) was constructed from 30 cows selected randomly, which had no genetic relationship, and was used as a template to amplify different regions of *STAT3*. DNA sequencing was carried out to screen variations within the amplified regions of the DNA pools constructed, and the products amplified from genomic DNA were directly sequenced in both directions. Sequences were analyzed with the DNASTAR software (Version.7.0).

Five SNPs (g.43591G>A, g.65812G>A, g.67492T>G, g.67519T>C, and g.68964G>A) were detected and are illustrated in Figure 1. According to the sequence mutations, the PCR products could be digested with *Lpn*p1, *Tai*1, *Bse*11, *Nco*1, and *Bsp*AC1 restriction enzymes to genotype the individuals. Aliquots of 10 μL PCR products were digested with 10 U *Lpn*p1

(g.43591G>A), *Tai*1 (g.65812G>A), *Bse*11 (g.67492T>G), *Nco*1 (g.67519T>C), and *Bsp*AC1 (g.68964G>A) for 5 h at 37°C, respectively. The digested products were detected by electrophoresis on a 2.5% agarose gel stained with ethidium bromide.

Table 1. Primers used to amplify bovine *STAT3*.

Amplified region	Primer		Tm (ºC)	Size (bp)
Exon 16	Primer A	F: 5'-CCCCTTGGATTGAGAGTC-3' R: 5'-CCTCTTTACTTTCCAATCTC-3'	51.6	175
Intron 13	Primer B	F: 5'-GTGAACTTTTTCACCAAACC-3' R: 5'-TTGCTCAGTCAACTTTCC-3'	57.6	221
Intron 19	Primer C	F: 5'-TGGCAGCCCCCATCAGAAC-3' R: 5'-GTATCCAGATTCCACCAGCAG-3'	55.9	436
Intron 20	Primer D	F: 5'-TGGCAGCCCCCATCAGAAC-3' R: 5'-GTATCCAGATTCCACCAGCAG-3'	55.0	382

Tm = melting temperature.

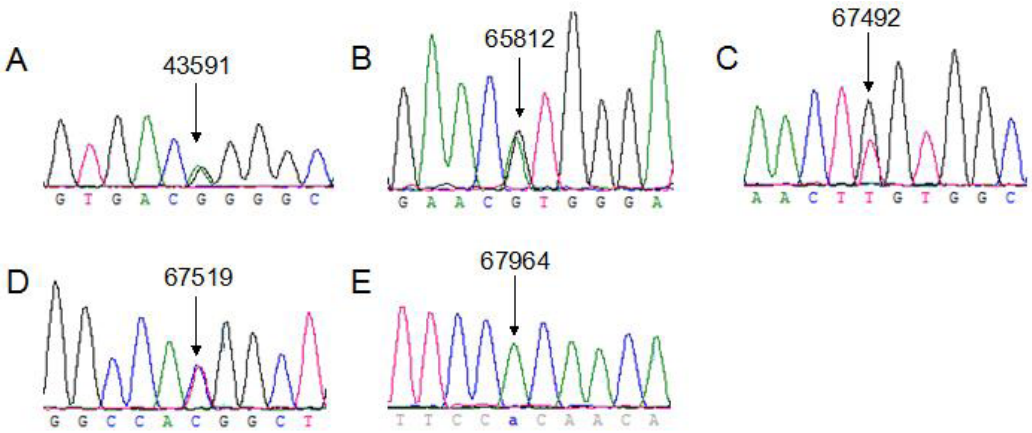

Figure 1. Sequencing maps of *STAT3* gene SNPs in beef cattle. **A.** g.43591G>A; **B.** g.65812G>A; **C.** g.67492T>G; **D.** g.67519T>C; **E.** g.68964G>A.

Statistical analysis

Genetic diversity parameters including genotype frequencies, heterozygosity (H_E), Hardy-Weinberg equilibrium (HWE), and polymorphism information content (PIC) were calculated using the Popgen32 software, and they are summarized in Table 2.

To investigate the association of *STAT3* genotypes with growth and carcass traits, general linear models were generated with the SPSS software (Version. 16.0). The following statistical linear model was used: Yi = μ + Gi + Ai + Eik, where *Yi* was the traits measured for each of the individual cattle, μ was the overall population mean for the traits, *Gi* was the fixed effect associated with the genotype, *Ai* was the fixed effect due to age, and *Eik* was the standard error.

Table 2. Genotype frequencies, H_E, HWE, and PIC of the *STAT3* gene SNPs in a Qinchuan cattle population.

SNPs	Breeds	No.	Frequencies			H_E	χ^2 (HWE)	PIC
g.43591G>A	QC	371	0.46 (GG)	0.28 (GA)	0.26 (AA)	0.48	>0.05	0.36
g.65812G>A			0.55 (GG)	0.35 (GA)	0.10 (AA)	0.40	4.18	0.32
g.67492T>G			0.38 (TT)	0.36 (TG)	0.26 (GG)	0.49	>0.05	0.37
g.67519T>C			0.12 (TT)	0.23 (TC)	0.65 (CC)	0.36	>0.05	0.29
g.67964G>A			0.54 (GG)	0.38 (GA)	0.08 (AA)	0.39	0.65	0.32
g.43591G>A	JX	122	0.60 (GG)	0.26 (GA)	0.14 (AA)	0.39	>0.05	0.32
g.65812G>A			0.52 (GG)	0.23 (GA)	0.25 (AA)	0.46	>0.05	0.35
g.67492T>G			0.32 (TT)	0.31 (TG)	0.37 (GG)	0.50	>0.05	0.37
g.67519T>C			0 (TT)	0.28 (TC)	0.72 (CC)	0.24	3.20	0.21
g.67964G>A			0.56 (GG)	0.44 (GA)	0 (AA)	0.34	>0.05	0.29

RESULTS

SNPs identified

Bovine *STAT3* is located on chromosome 19, and it contains 23 exons encoding 1386 amino acids. In the present study, 5 variations including 1 mutation in an exon (g.65812G>A: exon 16) and 4 non-coding mutations in an intron (g.43591G>A: 13 intron, g.67492T>G: 19 intron, g.67519T>C: 19 intron and g.68964G>A: 20 intron) were revealed by comparing the sequencing results with the DNA sequence of *STAT3* published in GenBank. PCR-RFLP and DNA sequencing were used for further genotyping.

g.43591G>A, genotyped by *Lpn*p1 endonuclease, showed one 175-bp fragment for GG, 3 fragments (175, 135, and 40 bp) for GA, and 2 fragments (135 and 40 bp) for AA. For g.65812G>A, digestion of the 148-bp PCR fragment with *Tai*1 resulted in a 221-bp product for the AA, 221, 187, and 34 bp for the GA, and 187 and 34 bp for the GG. For g.67492T>G, *Bse*11 digested 436-bp products and generated 1 fragment (436 bp) for the TT, 2 fragments (335 and 101 bp) for the GG, and 3 fragments (436, 335, and 101 bp) for TG. For g.67519T>C, the 436-bp products digested by *Nco*1 formed 1 fragment (436 bp) for TT, 2 fragments (364 and 72 bp) for CC, and 3 fragments (436, 364, and 72 bp) for TC. For g.68964G>A, the 382-bp products digested by *Bsp*AC1 formed 1 fragment (382 bp) for the GG, 2 fragments (340 and 42 bp) for AA, and 3 fragments (382, 340, and 42 bp) for GA.

Diversity analyses

The genotypic frequencies and genetic diversity parameters (H_E and PIC) of the 5 SNPs are presented in Table 2. The PIC was calculated for each locus, and the values ranged from 0.21 to 0.37. According to the PIC calculation, our data showed that the cattle populations had intermediate genetic diversity at the 5 SNP loci, except for Jiaxian cattle populations in g.67519T>C. This reflected the fact that there was not a very high genetic diversity within Chinese bovine *STAT3* in the analyzed populations.

The Chi-square test showed that neither g.65812G>A nor g.68964G>A followed HWE, whereas the individual frequencies of the genotypes agreed were in HWE for other SNPs in Qinchuan cattle populations. For the Jiaxian cattle, the genotype distributions of the 4 mutations were in HWE except the g.67519T>C.

Effect of the polymorphism locus on body measurement and carcass quality traits

The association between *STAT3* polymorphism and body measurement traits was analyzed, and the results are shown in Table 3. At the g.43591G>A locus, the mean value of animals with genotype AA was significantly different from that of animals with genotype GG for the parameters body length and backfat thickness ($P < 0.01$). Meanwhile, there was a significant difference between the AA and GG genotypes with withers height and chest circumference ($P < 0.05$). At the g.65812G>A locus, animals with genotype GA had significantly greater body length, chest circumference, and backfat thickness than those with genotype AA ($P < 0.05$). At the g.67492T>G locus, animals with genotype GG had significantly bigger loin muscle area as determined by ultrasound than those with genotype TT ($P < 0.01$). At the g.67492T>G locus, animals with genotype CC had significantly greater body length and chest circumference than those with genotype TT ($P < 0.05$). Significant differences were found in backfat thickness between the 2 genotypes ($P < 0.01$). At the g.68964G>A locus, animals with genotype GA had significantly greater body length than those with genotype AA ($P < 0.05$). For backfat thickness, the AG genotype had a higher mean value than the AA genotype did ($P < 0.01$).

Table 3. Association of SNP genotypes of the *STAT3* gene with body measurement traits in beef cattle.

Locus	Genotypes	BL (cm)	WH (cm)	CC (cm)	BT (cm)	ULA (cm^2)	IMF (cm)
g.43591G>A	GG (172)	137.21 ± 0.42^{Bb}	122.99 ± 0.62^{b}	43.19 ± 0.26^{b}	0.75 ± 0.01^{C}	63.49 ± 0.64	7.00 ± 0.06
	GA (103)	142.15 ± 0.54^{a}	125.30 ± 0.57	44.35 ± 0.33	0.91 ± 0.01^{B}	63.37 ± 0.81	7.12 ± 0.08
	AA (96)	145.82 ± 0.57^{A}	127.97 ± 0.60^{a}	45.00 ± 0.35^{a}	1.33 ± 0.02^{A}	65.38 ± 0.86	7.17 ± 0.09
	P	0.001	0.032	0.021	0.000	0.067	0.123
g.65812G>A	GG (205)	140.96 ± 0.44^{a}	125.14 ± 0.43	44.16 ± 0.24^{a}	0.95 ± 0.19^{a}	63.98 ± 1.57^{b}	7.15 ± 0.06
	GA (131)	141.94 ± 0.55^{a}	125.21 ± 0.54	44.06 ± 0.30^{a}	0.96 ± 0.24^{a}	68.93 ± 1.21^{a}	7.03 ± 0.07
	AA (35)	135.45 ± 1.07^{b}	122.43 ± 1.04	42.60 ± 0.58^{b}	0.82 ± 0.47^{b}	64.41 ± 2.22^{b}	6.98 ± 0.14
	P	0.036	0.157	0.036	0.011	0.018	0.212
g.67492T>G	TT (140)	139.69 ± 0.55	123.93 ± 0.52	43.35 ± 0.29	0.94 ± 0.02	63.67 ± 1.10^{B}	7.04 ± 0.07
	TG (133)	141.08 ± 0.56	125.64 ± 0.53	44.07 ± 0.31	0.93 ± 0.02	63.64 ± 1.18^{B}	7.17 ± 0.07
	GG (98)	141.97 ± 0.66	123.93 ± 0.62	44.76 ± 0.35	0.95 ± 0.03	71.65 ± 2.71^{A}	7.04 ± 0.09
	P	0.422	0.123	0.068	0.087	0.000	0.211
g.67519T>C	TT (44)	137.34 ± 0.97^{b}	123.19 ± 0.93	42.66 ± 0.52^{b}	0.75 ± 0.04^{B}	62.63 ± 1.26	7.02 ± 0.13
	TC (85)	140.38 ± 0.69	124.35 ± 0.66	44.02 ± 0.37	0.90 ± 0.03^{A}	64.55 ± 1.11	7.17 ± 0.09
	CC (242)	141.57 ± 0.41^{a}	125.41 ± 0.39	44.20 ± 0.22^{a}	0.99 ± 0.03^{A}	63.96 ± 0.84	7.07 ± 0.06
	P	0.041	0.288	0.029	0.009	0.064	0.333
g.67964G>A	GG (201)	140.41 ± 0.46	124.73 ± 0.43	44.12 ± 0.24	0.90 ± 0.02^{a}	64.63 ± 0.59	7.13 ± 0.06
	GA (140)	142.04 ± 0.55^{a}	125.63 ± 0.58	43.98 ± 0.29	1.02 ± 0.02^{A}	63.40 ± 0.70	7.05 ± 0.07
	AA (30)	137.55 ± 1.18^{b}	122.73 ± 1.11	43.00 ± 0.63	0.80 ± 0.05^{Bb}	61.79 ± 1.52	6.92 ± 0.16
	P	0.029	0.320	0.117	0.000	0.072	0.291

^{a,b}Means with different superscripts are significantly different ($P < 0.05$). ^{A,B}Means with different superscripts are significantly different ($P < 0.01$). BL = body length, WH = withers height, CC = chest circumference, BT = backfat thickness, ULA = ultrasound loin muscle area, and IMF = intramuscular fat.

DISCUSSION

During bovine breeding, body measurement and carcass traits are considered economically important, and are affected by many factors such as genotype, sex, age, breed, herd, location, and other random environment factors (Gui et al., 2014). Over the past 40 years, genetic improvement has been achieved by selection based on phenotypic information (Zhang et al., 2008). Presently, many genes have been identified to be involved in controlling growth (Liu et al., 2012b; Zhang et al., 2012), reproduction (Chu et al., 2010), and meat quality (Jiao et al., 2010; Li et al., 2013) in livestock. In addition, the STAT protein family is a group of

transcription factors that are found in diverse organisms, ranging from flatworms to humans. In particular, STAT3 regulates diverse target pathways involved in bone formation (Zhou et al., 2011) and adipocyte differentiation (Priceman et al., 2013), which is important for body measurement and carcass quality traits in livestock.

The objective of the present study was to identify and characterize polymorphisms within the coding and non-coding regions of bovine *STAT3* in 371 individual Qinchuan cattle. These SNPs (g.43591G>A, g.65812G>A, g.67492T>G, g.67519T>C and g.68964G>A) were detected by PCR-RFLP and DNA sequencing. Our results showed that g.43591G>A is associated with body length, withers height, chest circumference, and backfat thickness, and AA appears to be the beneficial genotype; both g.65812G>A and g.67519T>C are associated with body length, chest circumference, and backfat thickness, and the GG and CC genotype seems to be beneficial, respectively; g.68964G>A is associated with body length and backfat thickness, and GA seems to be the beneficial genotype. Based on the results, we suggest that STAT3 has a potential effect on body measurement and carcass quality traits in Qinchuan cattle population.

Here, g.43591G>A, g.67492T>G, g.67519T>C, and g.68964G>A were identified in introns, and did not change the structure of the encoded proteins. Recently, however, studies have shown that intronic mutations may affect splice sites and consequently mRNA stability, and may lead to truncated or a lack of protein products (Ibeagha-Awemu et al., 2008). Importantly, further studies are needed to determine how the mutations affect phenotypic variation of these traits.

In conclusion, to our knowledge, this study is the first report of 5 SNPs in bovine *STAT3*. We analyzed their association with body measurements and carcass quality traits in a Qinchuan cattle population. Based on these results, it can be inferred that mutations in *STAT3* affect economic traits and might be used as genetic marker for breeding beef cattle.

Conflicts of interest

The authors declare no conflict of interest.

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