Polymorphism identification in goat *DGAT1* and *STAT5A* genes and association with milk production traits

X.P. An, J.X. Hou, H.B. Zhao, L. Bai, J.Y. Peng, C.M. Zhu, Q.M. Yan, Y.X. Song, J.G. Wang, B.Y. Cao

College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, P.R. China

ABSTRACT: **P**olymorphisms of *DGAT1* and *STAT5A* genes in Xinong Saanen and Guanzhong goat breeds were investigated. PCR-RFLP, SSCP, and DNA sequencing were used to identify three SNPs: DQ380250:g.407_408*ins*C in the *DGAT1* gene, AJ237937:g.6798C>T and g.6852C>T in the *STAT5A* gene. In *DGAT1 g.407_408insC* locus, the frequencies of C^- allele were 0.79–0.85, and frequencies of C^+ allele were 0.21–0.15. At *STAT5A g.6852C>T* locus, frequencies of *C* allele were 0.70–0.72, and frequencies of *T* allele were 0.30–0.28. Compared with goats with *DGAT1* C^-C^-, those with C^-C^+ genotype had greater milk fat ($P < 0.05$). The goats with *STAT5A CT* had greater milk yield than those with *CC* genotype ($P < 0.05$). The results showed that does with C^-C^-CT and C^-C^+CT yielded more milk than those with C^-C^-CC ($P < 0.05$). In addition, does with C^-C^+CT had the highest milk fat in comparison with other combination genotypes ($P < 0.05$).

Keywords: milk yield; fat percentage; dairy goat; SNP; PCR-RFLP; SSCP; additive effect

Milk production traits are of fundamental importance in livestock production and the related economy (Erhardt et al., 2010; An et al., 2011). Selection aimed at increasing the frequency of alleles with a positive effect on a given trait was initiated by geneticists (Dekkers, 2004). Variations in these genes, which show associations with specific economically important traits, are useful markers for marker-assisted selection (Parmentier et al., 1999). In general, identifying and validating genetic markers for milk production traits is the initial and crucial step to establish a MAS system.

Diacylglycerol acyltransferases (DGATs) catalyze the final step of the triacylglycerol (TAG) biosynthesis of the Kennedy pathway (Hatzopoulos et al., 2011). Two genes (*DGAT1* and *DGAT2*) have been shown to encode DGATs. Both genes encode membrane-bound proteins, with no sequence homology to each other (Giannoulia et al., 2000). *DGAT1* gene was the first identified gene encoding a protein with DGAT activity (Cases et al., 2001). Diacylglycerol acyltransferase 1 (*DGAT1*) was identified as one underlying quantitative trait locus (QTL) for milk production traits in the centromeric region of the bovine chromosome 14 (Grisart et al., 2002; Winter et al., 2002). By sequencing the *DGAT1* gene from animals with known QTL genotypes, a nonconservative lysine to alanine substitution was identified at position 232, and shown to be associated with a major effect on milk yield and composition in several dairy cattle breeds (Grisart et al., 2002; Spelman et al., 2002; Winter et al., 2002). The signal transducers and activators of transcription (STATs), a family of transcription factors, mediate the actions of a variety of peptide hormones and cytokines (Darnell et al., 1994; Darnell, 1997). STAT5, also known as mammary gland factor (MGF), was discovered initially as a

Supported by the National Support Program of China (Project No. 2011BAD28B05-3) and the Science and Technology Innovation Project of Shaanxi Province (Project No. 2011KTCL02-09).

PRL-induced transcription factor (Wakao et al., 1994). It is a key intracellular mediator of prolactin signaling and can activate transcription of milk protein genes in response to prolactin (Wakao et al., 1994). STAT5 exists in two isoforms – A and B, which differ by a few amino acids in the carboxylic end of the protein molecule; separate genes code both of them (Seyfert et al., 2000). In goat, the *STAT5A* gene was located at chromosome 19 (Goldammer et al., 1997). Antoniou et al. (1999) described two SSCP variants of the gene fragment that encodes the SH2 domain in bovine STAT5A protein. Brym et al. (2004) detected a new SNP (A/G) located in intron 9 of *STAT5A* gene at position 9501. Schennink et al. (2009) demonstrated that g.9501G>A base mutation in *STAT5A* gene showed significant effects on milk-fat composition. Sadeghi et al. (2009) studied the association between this polymorphism of *STAT5A* gene and the breeding values of milk production traits in 134 Iranian Holstein bulls. He et al. (2012) reported that 17266indelCCT polymorphic site of *STAT5A* gene was a potential DNA marker for improving milk yield and fat percentage in dairy cattle. The aims of this study were to investigate SNPs in *DGAT1* and *STAT5A* genes in goats, and analyze their association with milk production traits.

MATERIAL AND METHODS

Animals and genomic DNA isolation

Blood samples were obtained from 528 goats belonging to two breeds: Xinong Saanen (SN, n = 285) and Guanzhong (GZ, n = 243) reared in Qianyang county and Zhouzhi county of Shaanxi Province, respectively. They were reared under the same dry-lot nutrition standard conditions. All studied animals came from 11 Xinong Saanen and 10 Guanzhong sires. The number of grand parents and parents was 108 and 180, respectively. The number of daughters of the Xinong Saanen sires ranged 15–37, while that of the Guanzhong sires ranged 15–34. There were no records of female grand-grand parents. Health, fertility, and milk recording was carried out by dairymen and veterinarians. Data was recorded in winter and spring parturitions of 2008–2011. Milk yields from the first to the third lactation were standardized to 300 days in milk. For milk analysis, a milk sample was taken from each animal once per month throughout the third lactation, sampling first at least 20 days after parturition to exclude the risk of contamination with colostrum. Goats were milked twice a day at constant intervals and a 10 ml sample from each milking session was mixed for the analysis. Milk constituents (protein, lactose, and fat) were determined with an ultrasonic S60SEC milk analyzer (Milkotronic Ltd., Nova Zagora, Bulgaria). 5 ml blood per goat were collected aseptically from the jugular vein and kept in a tube containing anticoagulant ACD (citric acid : sodium citrate : dextrose – 10 : 27 : 38). All samples were delivered back to the laboratory in an ice box. The genomic DNA was extracted from white blood cells using standard phenol-chloroform extraction protocol (Mullenbach et al., 1989).

PCR amplification

According to bovine *DGAT1* and *STAT5A* genes (GenBank accession Nos. AJ318490 and AJ237937), fourteen pairs of primers were designed to amplify goat *DGAT1* and *STAT5A* genes. Pairs of primers 1 and 2 are shown in Table 1. Other primer pairs with no polymorphism detected in their amplification regions are not listed. The 25 µl volume contained 50 ng genomic DNA, 12.5 µl 2X reaction mix (including 500µM dNTP each; 20mM Tris-HCl; pH 9; 100mM KCl; 3mM $MgCl_2$), 0.5µM of each primer, and 0.5 units of *Taq* DNA polymerase. The cycling protocol was 5 min at 95°C, 35 cycles of denaturing at 94°C for 30 s, annealing at 59°C (primer pair 1) and 63°C (primer pair 2) for 30 s, extending at 72°C for 30 s, with a final extension at 72°C for 10 min.

SNP genotyping and sequencing

SSCP analysis: PCR products (5 µl) of *DGAT1* obtained with primer pair 1 were mixed with 5 µl of denaturing solution (95% formamide, 25mM EDTA, 0.025% xylene-cyanole, and 0.025% bromophenol blue), heated at 98°C for 10 min and chilled on ice. Denatured DNA samples were subjected to 10% PAGE (80 × 73 × 0.75 mm) in 1X TBE buffer at constant voltage (190 V) and temperature (4°C) for 4.5 h.

PCR-RFLP analysis: PCR products (5 µl) of *STAT5A* obtained with primer pair 2 were digested with 3 U of *Eco*81I (TaKaRa, Dalian, China) at 37°C for 1.5 h. Digested products were subjected to 12%

PAGE (80 × 73 × 0.75 mm) in 1X TBE buffer and constant voltage (110 V) for 1.5 h. The gel (29 : 1 acrylamide : bis) was stained with 0.1% silver nitrate (Ji et al., 2007). After the polymorphisms were detected, amplicons representing unique banding patterns were sequenced in both directions in ABI 377 DNA analyzer (Applied Biosystems, Foster, USA) and the sequences were analyzed with DNA-STAR software (Version 7.1, 2010) and BLAST software (Version 2.2.25, 2011) in the National Center for Biotechnology Information.

Statistical analysis

The allelic frequencies, heterozygosity (He), and polymorphism information content (PIC) were calculated using Cluster analysis software (Version 1.2, 2003). Milk production traits analyzed in the current study included milk yield, milk protein, lactose, and fat. Statistical analysis was performed using univariate analysis in the General Linear Model procedure of SPSS (Version 16.0, 2010) statistical software. The linear model applied was:

$$Y_{ijklmn} = \mu + G_i + B_j + P_k + N_l + (GP)_{ik} + S_m + E_{ijklmn} \quad (1)$$

where:

Y_{ijklmn} = trait measured on each of the $ijklmn^{th}$ animal
μ = overall population mean
G_i = fixed effect associated with the i^{th} genotype
B_j = fixed effect associated with the j^{th} breed
P_k = fixed effect associated with the k^{th} parity
N_l = fixed effect associated with the l^{th} number of kids born
$(GP)_{ik}$ = interaction between the i^{th} genotype and the k^{th} parity
S_m = random effect associated with the m^{th} sire
E_{ijklmn} = random error

The combined effects of *DGAT1* and *STAT5A* genes on milk production traits were analyzed with the following model:

$$Y_{ijklmn} = \mu + C_i + B_j + P_k + N_l + (CP)_{ik} + S_m + E_{ijklmn} \quad (2)$$

where:

Y_{ijklmn}, μ, B_j, P_k, N_l, S_m, E_{ijklmn} = like in model (1)
C_i = fixed effect associated with the i^{th} combination genotype
$(CP)_{ik}$ = interaction between the i^{th} combination genotype and the k^{th} parity

Effects associated with farm, birth year, and season of birth are not matched in the linear model, as the preliminary statistical analyses indicated that these effects did not have a significant influence on variability of traits in the analyzed populations.

RESULTS

SNPs identification and genotypes

Polymorphisms detected in PCR fragments of the goat *DGAT1* and *STAT5A* genes are shown in Figure 1. In Figure 1A, SSCP analysis of *DGAT1* SNP DQ380250:g.407_408*ins*C is shown (C^- – allele with deletion, C^+ – allele with insertion) while Figure 1B shows PCR-RFLP analysis of *STAT5A* SNP AJ237973:g.6852C>T, after digestion with *Eco*81I (allele *C* – 162 + 53 bp; allele *T* – 126 + 53 + 36 bp). The SNPs were identified by sequencing of the PCR fragments. The representative sequences have been deposited in GenBank database under accession numbers JF781126 (*DGAT1*) and JN091564 (*STAT5A*). The *DGAT1* SNP is located in intron 14 and *STAT5A* SNP is present in exon 7. In this exon, another SNP was also identified (g.6798C>T). Only

Table 1. Primer sequences and information on goat *DGAT1* and *STAT5A* genes

Gene	Primer	Sequence (bp)	T_a (°C)	Amplicon	Product size (bp)
DGAT1	P1	F: 5'-AGGAACTCGGAGTCCATCAC-3' R: 5'-TGAAGGCCCAGAGGCGGAAC-3'	59	exon 14–16	328
STAT5A	P2	F: 5'-CTGCAGGGCTGTTCTGAGAG-3' R: 5´-TGGTACCAGGACTGTAGCACAT-3'	63	exon 7	215

T_a = annealing temperature

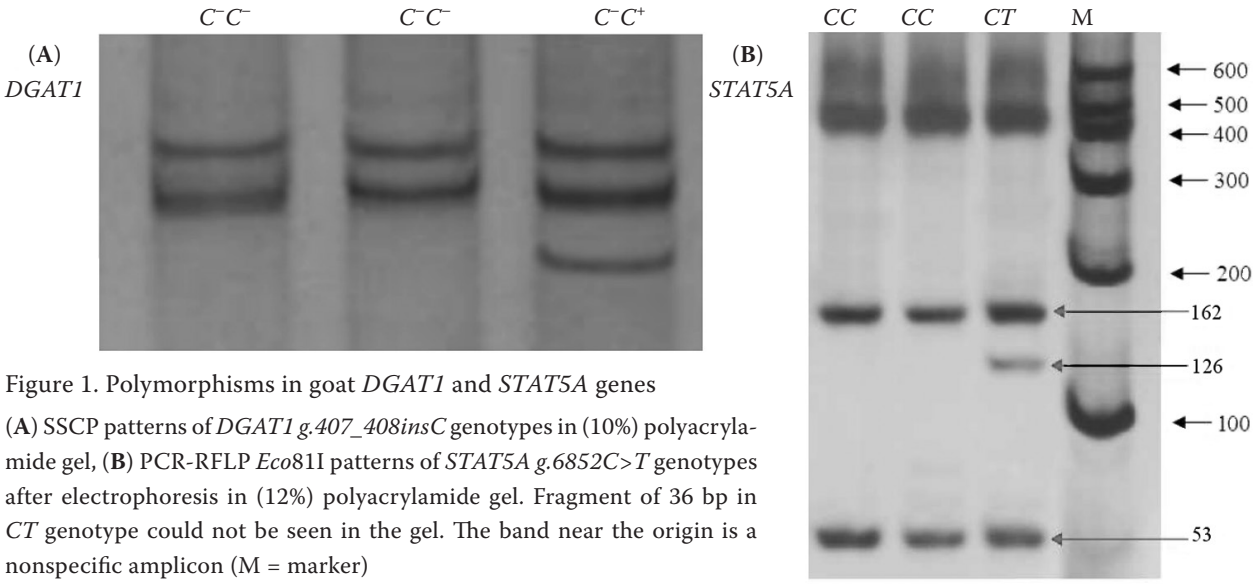

Figure 1. Polymorphisms in goat *DGAT1* and *STAT5A* genes

(**A**) SSCP patterns of *DGAT1 g.407_408insC* genotypes in (10%) polyacrylamide gel, (**B**) PCR-RFLP *Eco*81I patterns of *STAT5A g.6852C>T* genotypes after electrophoresis in (12%) polyacrylamide gel. Fragment of 36 bp in *CT* genotype could not be seen in the gel. The band near the origin is a nonspecific amplicon (M = marker)

heterozygotes (n = 528) were found in *g.6798C>T* locus by PCR-SSCP and DNA sequencing, so it was not included in the further analysis. Both *STAT5A* mutations are synonymous.

Allelic frequencies and additive effect of two SNP loci

Allelic frequencies, He, and PIC are shown in Table 2. We found that the additive effect of *STAT5A* and *DGAT1* SNPs on milk yield and fat percentage was highly significant ($P < 0.001$), respectively. The additive effect between *DGAT1* and *STAT5A* genes had highly significant effects on milk fat percentage ($P < 0.001$) (Table 3).

Table 2. Genotype distribution and allele frequencies of *DGAT1 g.407_408insC* and *STAT5A g.6852C>T* loci in Xinong Saanen (SN) and Guanzhong (GZ) goat breeds

Locus			Breed	
			SN	GZ
g.407_408insC	genotype	C^-C^-	197	141
		C^-C^+	88	102
	allele	C^-	0.85	0.79
		C^+	0.15	0.21
	He		0.31	0.42
	PIC		0.23	0.28
g.6852C>T	genotype	*CC*	112	106
		CT	173	137
	allele	*C*	0.70	0.72
		T	0.30	0.28
	He		0.61	0.56
	PIC		0.33	0.32

He = heterozygosity, PIC = polymorphism information content

Association of SNPs with milk production traits

At *g.407_408insC* and *g.6852C>T* loci, the animal genotypes were analyzed for association with milk yield and constituents, respectively in SN and GZ breeds (Table 4). Milk protein and lactose did not show any significant association with genotypes. At *g.407_408insC* locus, the does with C^-C^+ genotype had greater milk fat than those with C^-C^- genotype in SN and GZ breeds ($P < 0.05$). At *g.6852C>T* locus, the does with *CT* genotype had greater milk yield than those with *CC* genotype ($P < 0.05$) in SN and GZ breeds (Table 4). In addition, the study analyzed both breeds together for associations (Table 4). These results were consistent with single breed association study. The does with C^-C^-CT and C^-C^+CT had higher milk yield than those with C^-C^-CC ($P < 0.05$) in SN and GZ breeds (Table 5). In addition, the does with C^-C^+CT had the highest milk fat in comparison with other combination genotypes ($P < 0.05$) in SN breed. Table 5 shows the association analysis results of both breeds together. In comparison with other combination genotypes (Table 5), the does with C^-C^+CT had the highest milk fat ($P < 0.05$).

Table 3. Additive effect of *DGAT1 g.407_408insC* and *STAT5A g.6852C>T* loci on milk yield and fat percentage

Locus	Effect	Milk yield (kg)	Milk fat (%)
g.407_408insC	additive	−1.58 ± 4.93	0.15 ± 0.03
	P-value	0.75	< 0.001
g.6852C>T	additive	18.78 ± 10.08	0.03 ± 0.06
	P-value	< 0.001	0.36
g.407_408insC and *g.6852C>T*	additive × additive	−7.80 ± 2.36	0.18 ± 0.02
	P-value	0.43	< 0.001

DISCUSSION

The results of this study showed that the C^+ (*g.407_408insC*) and *T* (*g.6852C>T*) alleles had low frequencies (0.15–0.30), and C^+C^+ and *TT* genotypes were not observed, respectively in SN and GZ breeds. We consider that the results can be explained by the following two reasons: (1) There is a lower frequency of missing genotypes. (2) The missing genotypes of the two loci have negative effects on individual performance, so the animals with missing genotypes have been eliminated in breeding process.

We firstly revealed the significant association of *DGAT1 g.407_408insC* and *STAT5A g.6852C>T* loci with milk yield and fat in dairy goats ($P < 0.05$). Although the mutations of *g.407_408insC* and *g.6852C>T* loci do not concern the coding region and the change of amino acid, they possibly influence the stability of the mRNA and the mechanism of mRNA deadenylation. Linkage disequilibrium with the causal mutation possibly affects the variation of milk production traits in goat (Van der Werf et al., 2007). Amills et al. (2007) indicated the T to C substitution in intron 16 of goat *DGAT1* gene could be used as a marker in association studies with milk traits. Flisikowski et al. (2004) showed the T→C transition at position 12743 in exon 16 of *STAT5A* gene had an effect on milk yield and composition in Polish Black-and-White cows. Selvaggi et al. (2009) reported a substitution C→T at position 6853 of *STAT5A* gene led to three genotypes (*CC*, *CT*, and *CT*), and the cows with *CC* genotype had higher milk yield and

Table 4. Association analysis of *DGAT1 g.407_408insC* and *STAT5A g.6852C>T* loci with milk yield and constituents in Xinong Saanen (SN) and Guanzhong (GZ) goats

Breed	Gene	Genotype	Milk yield (kg)	Milk fat (%)	Milk protein (%)	Lactose (%)
SN	*DGAT1*	C^-C^- (197)	653.88 ± 2.75	3.37 ± 0.02[a]	2.95 ± 0.01	4.44 ± 0.02
		C^-C^+ (88)	658.75 ± 4.11	3.52 ± 0.04[b]	2.98 ± 0.01	4.43 ± 0.03
	STAT5A	*CC* (112)	646.47 ± 3.63[a]	3.39 ± 0.03	2.95 ± 0.01	4.40 ± 0.02
		CT (173)	661.16 ± 2.92[b]	3.44 ± 0.03	2.97 ± 0.01	4.45 ± 0.02
GZ	*DGAT1*	C^-C^- (141)	654.73 ± 3.11	3.40 ± 0.03[a]	3.01 ± 0.01	4.47 ± 0.03
		C^-C^+ (102)	655.19 ± 3.66	3.55 ± 0.04[b]	3.03 ± 0.02	4.45 ± 0.03
	STAT5A	*CC* (106)	639.36 ± 3.50[a]	3.45 ± 0.04	3.02 ± 0.02	4.46 ± 0.03
		CT (137)	666.96 ± 3.08[b]	3.47 ± 0.03	3.03 ± 0.01	4.46 ± 0.03
SN + GZ	*DGAT1*	C^-C^- (338)	653.71 ± 2.25	3.38 ± 0.03[a]	2.97 ± 0.01	4.46 ± 0.02
		C^-C^+ (190)	660.29 ± 3.25	3.48 ± 0.03[b]	2.96 ± 0.01	4.45 ± 0.02
SN + GZ	*STAT5A*	*CC* (218)	642.22 ± 3.06[a]	3.41 ± 0.03	2.97 ± 0.01	4.47 ± 0.02
		CT (310)	665.67 ± 2.48[b]	3.45 ± 0.03	2.96 ± 0.01	4.45 ± 0.01

numbers in brackets indicate the number of samples, milk samples from the third lactation were analyzed for milk constituents
data are expressed as Least Squares Means ± standard errors
[a,b]values within the same column in particular population differ significantly at $P < 0.05$

Table 5. Association of *DGAT1* and *STAT5A* genes with milk yield and constituents in Xinong Saanen (SN) and Guanzhong (GZ) goats

Breed	Genotypic combination	Milk yield (kg)	Milk fat (%)	Milk protein (%)	Lactose (%)
SN	*C⁻C⁻CC* (79)	643.81 ± 4.32[a]	3.39 ± 0.04[a]	2.96 ± 0.02	4.40 ± 0.02
	C⁻C⁺CC (33)	652.86 ± 6.69	3.38 ± 0.06[a]	2.96 ± 0.03	4.38 ± 0.04
	C⁻C⁻CT (118)	660.63 ± 3.54[b]	3.36 ± 0.03[a]	2.99 ± 0.01	4.44 ± 0.02
	C⁻C⁺CT (55)	662.28 ± 5.18[b]	3.61 ± 0.04[b]	3.01 ± 0.02	4.43 ± 0.03
GZ	*C⁻C⁻CC* (68)	639.98 ± 4.38[a]	3.42 ± 0.04[a]	2.98 ± 0.02	4.47 ± 0.04
	C⁻C⁺CC (38)	638.25 ± 5.86[a]	3.51 ± 0.06	2.97 ± 0.02	4.45 ± 0.05
	C⁻C⁻CT (73)	668.47 ± 4.23[b]	3.38 ± 0.04[a]	3.01 ± 0.02	4.45 ± 0.03
	C⁻C⁺CT (64)	665.24 ± 4.51[b]	3.57 ± 0.05[b]	3.01 ± 0.02	4.43 ± 0.04
SN + GZ	*C⁻C⁻CC* (147)	642.10 ± 3.65[a]	3.44 ± 0.03[a]	2.96 ± 0.01	4.46 ± 0.03
	C⁻C⁺CC (70)	645.23 ± 5.48	3.41 ± 0.05[a]	2.97 ± 0.02	4.45 ± 0.04
	C⁻C⁻CT (191)	664.35 ± 3.32[b]	3.38 ± 0.03[a]	2.95 ± 0.01	4.42 ± 0.02
	C⁻C⁺CT (120)	666.21 ± 3.89[b]	3.59 ± 0.04[b]	2.93 ± 0.01	4.47 ± 0.03

numbers in brackets indicate the number of samples, milk samples from the third lactation were analyzed for milk constituents
data are expressed as Least Squares Means ± standard errors
[a,b]values within the same column in particular population differ significantly at $P < 0.05$

protein content than those with *CT* genotype. The biochemical and physiological functions, together with the results obtained in our study, indicate that the goat *DGAT1* and *STAT5A* genes might play important roles in affecting milk production traits.

Additive effect could be truly transmitted to offspring and this is the focus of marker-assisted selection. In this study, we took into account additive effect between SNP loci and milk production traits. The result showed the additive effect of *STAT5A g.6852C>T* and *DGAT1 g.407_408insC* loci on milk yield and fat was highly significant ($P < 0.001$), respectively. Compared with single SNP analysis, combination genotypes analysis provides more information on gene interactions. Multiple locus analysis used in the study revealed that the combined effect of *DGAT1 g.407_408insC* and *STAT5A g.6852C>T* loci significantly affected milk yield and fat. Based on the above considerations, we thought milk production traits were subjected to the impacts of *DGAT1 g.407_408insC* and *STAT5A g.6852C>T* loci, and there was an interaction between both loci.

CONCLUSION

Significant association between *DGAT1 g.407_408insC* and *STAT5A g.6852C>T* loci with milk yield and fat was disclosed in two Chinese dairy goats. Combination genotypic analysis further confirmed the significant effect of the two genes. These results implied that the *DGAT1* and *STAT5A* genes could be powerful candidate genes or linked to major genes that affect milk production traits in goats.

REFERENCES

Amills M., Angiolillo A., Urrutia B., Domenech A., Sastre Y., Badaoui B., Jordana J. (2007): Identification of a single nucleotide polymorphism at intron 16 of the caprine acyl-coenzyme A: Diacylglycerol acyltransferase 1 (*DGAT1*) gene. Journal of Dairy Research, 74, 47–51.

An X.P., Song S.G., Hou J.X., Zhu C.M., Peng J.X., Liu X.Q., Liu H.Y., Xiao W.P., Zhao H.P., Bai L., Wang J.G., Song Y.X., Cao B.Y. (2011): Polymorphism identification in goat *DGAT2* gene and association analysis with milk yield and fat percentage. Small Ruminant Research, 100, 107–112.

Antoniou E., Hirts B.J., Grosz M., Skidmorec J. (1999): A single strand conformation polymorphism in the bovine gene *STAT5A*. Animal Genetics, 30, 225–244.

Brym P., Kamiński S., Ruść A. (2004): New SSCP polymorphism within bovine *STAT5A* gene and its associations with milk performance traits in Black-and-White and Jersey cattle. Journal of Applied Genetics, 45, 445–452.

Cases S., Stone S.J., Zhou P., Yen E., Tow B., Lardizabal K.D., Voelker T., Farese R.V. (2001): Cloning of *DGAT2*,

a second mammalian diacylglycerol acyltransferase and related family members. Journal of Biological Chemistry, 276, 38870–38876.

Darnell J.E. (1997): STATs and gene regulation. Science, 277, 1630–1635.

Darnell J.E., Kerr I.M., Stark G.R. (1994): Jak-Stat pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science, 264, 1415–1421.

Dekkers J.C. (2004): Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. Journal of Animal Science, 82, E313–E328.

Erhardt G., Caroli A., Rizzi R., Luhken G. (2010): Milk protein genetic variation and casein haplotype structure in the Original Pinzgauer cattle. Journal of Dairy Science, 93, 1260–1265.

Flisikowski K., Strzałkowska N., Słoniewski K., Krzyżewki J., Zwierzchowski L. (2004): Association of a sequence nucleotide polymorphism in exon 16 of the *STAT5A* gene with milk production traits in Polish Black-and-White (Polish Friesian) cows. Animal Science Papers and Reports, 22, 515–522.

Giannoulia K., Haralampidis K., Poghosyan Z., Murphy D.J., Hatzopoulos P. (2000): Differential expression of diacylglycerol acyltransferase (*DGAT*) genes in olive tissues. Biochemical Society Transactions, 28, 695–697.

Goldammer T., Meyer L., Seyfert H.M., Brunner R.M., Schwerin M. (1997): *STAT5A* encoding gene maps to chromosome 19 in cattle and goat and to chromosome 11 in sheep. Mammalian Genome, 8, 705–706.

Grisart B., Coppieters W., Farnir F., Karim L., Ford C., Berzi P., Cambisano N., Mni M., Reid S., Simon P., Spelman R., Georges M., Snell R. (2002): Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine *DGAT1* gene with major effect on milk yield and composition. Genome Research, 12, 222–231.

Hatzopoulos P., Banilas G., Karampelias M., Makariti I., Kourti A. (2011): The olive *DGAT2* gene is developmentally regulated and shares overlapping but distinct expression patterns with *DGAT1*. Journal of Experimental Botany, 62, 521–532.

He X., Chu M.X., Qiao L., He J.N., Wang P.Q., Feng T., Di R., Cao G.L., Fang L., An Y.F. (2012): Polymorphisms of *STAT5A* gene and their association with milk production traits in Holstein cows. Molecular Biology Reports, 39, 2901–2907.

Ji Y.T., Qu C.Q., Cao B.Y. (2007): An optimal method of DNA silver staining in polyacrylamide gels. Electrophoresis, 28, 1173–1175.

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

Parmentier I., Portetellea D., Gengler N., Prandic A., Bertozzia C., Vleuricka L., Gilsond R., Renaville R. (1999): Candidate gene markers associated with somatotropic axis and milk selection. Domestic Animal Endocrinology, 17, 139–148.

Sadeghi M., Shahrbabak M.M., Mianj G.R., Javaremi A.N. (2009): Polymorphism at locus of *STAT5A* and its association with breeding values of milk production traits in Iranian Holstein bulls. Livestock Science, 123, 97–100.

Schennink A., Bovenhuis H., Leon-Kloosterziel K.M., van Arendonk J.A.M., Visker M. (2009): Effect of polymorphisms in the *FASN*, *OLR1*, *PPARGC1A*, *PRL* and *STAT5A* genes on bovine milk-fat composition. Animal Genetics, 40, 909–916.

Selvaggi M., Dario C., Normanno G., Celano G.V., Dario M. (2009): Genetic polymorphism of STAT5A protein: relationships with production traits and milk composition in Italian Brown cattle. Journal of Dairy Research, 76, 441–445.

Seyfert H.M., Pitra C., Meyer L., Brunner R.M., Wheeler T.T., Molenaar A., McCracken J.Y., Herrmann J., Thiesen H.J., Schwerin M. (2000): Molecular characterization of *STAT5A*- and *STAT5B*-encoding genes reveals extended intragenic sequence homogeneity in cattle and mouse and different degrees of divergent evolution of various domains. Journal of Molecular Evolution, 50, 550–561.

Spelman R.J., Ford C.A., McElhinney P., Gregory G.C., Snell R.G. (2002): Characterization of the *DGAT1* gene in the New Zealand dairy population. Journal of Dairy Science, 85, 3514–3517.

Van der Werf J.H.J., Marshall K., Lee S. (2007): Methods and experimental designs for detection of QTL in sheep and goats. Small Ruminant Research, 70, 21–31.

Wakao H., Gouilleux F., Groner B. (1994): Mammary-gland factor (Mgf) is a novel member of the cytokine regulated transcription factor gene family and confers the prolactin response. EMBO Journal, 13, 2182–2191.

Winter A., Kramer W., Werner F.A.O., Kollers S., Kata S., Durstewitz G., Buitkamp J., Womack J.E., Thaller G., Fries R. (2002): Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl-CoA : diacylglycerol acyltransferase (*DGAT1*) with variation at a quantitative trait locus for milk fat content. Proceedings of the National Academy of Sciences, 99, 9300–9305.

Received: 2012–02–28
Accepted after corrections: 2013–01–21

Corresponding Author

Yu Xuan Song, Ph.D., Northwest A&F University, College of Animal Science and Technology, No. 22 Xinong Road, Yangling, Shaanxi 712100, P.R. China
Tel.: +86 29 87 092 102, fax +86 29 87 092 164, e-mail: syx98728@163.com