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Mutations in Caprine DGAT1 and STAT5A Genes were Associated with Milk Production Traits

——Combined Effects of DGAT1 and STAT5A Genes on Milk Yield and Fat

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ABSTRACT

In this study, polymorphisms of the DGAT1 and STAT5A genes were detected in 528 individuals from Xinong Saanen and Guanzhong goat breeds by PCR-RFLP, PCR-SSCP and DNA sequencing methods. Three allelic variants were identified: DQ380250: g.407_408insC, AJ237937: g.6798C>T and g.6852C>T in both breeds. At g.407_408insC locus, the frequencies of C_1 allele were 0.79–0.85, and frequencies of C_2 allele were 0.21–0.15. At g.6852C>T locus, frequencies of C_3 allele were 0.70–0.72, and frequencies of T_3 allele were 0.30–0.28. Compared with goats with C_1C_1 and C_3C_3, those with C_1C_2 and C_3T_3 genotypes had significant effects on milk yield and fat percentage ($P<0.05$), respectively. The result showed that does with $C_1C_1C_3T_3$ and $C_1C_2C_3T_3$ had higher milk yield than those with $C_1C_2C_3C_3$ ($P < 0.05$). In addition, the combined effect of $C_1C_2C_3T_3$ on milk fat percentage was the highest in comparison with other combination genotypes ($P<0.05$).

Keywords: Dairy Goat; Milk Production Traits; Fat Percentage; Pedigree

1. Introduction

Milk production traits are of fundamental importance in livestock production and the related economy [1]. Selection aimed at increasing the frequency of alleles with a positive effect on a given trait was initiated by geneticists [2]. Meanwhile, variation of either candidate genes for production traits or linked genetic markers has informed the basic biology of milk production and composition, and encouraged the use of gene for marker assisted selection (MAS) in livestock [3]. 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) catalyse the final step of the triacylglycerol (TAG) biosynthesis of the Kennedy pathway [4]. Two genes (DGAT1 and DGAT2) have been shown to encode DGATs. Both genes encode membrane-bound proteins, with no sequence homology to each other [5]. *DGAT1* gene was the first identified gene encoding a protein with DGAT activity [6]. Diacylglycerol acyltransferase1 (DGAT1) was identified as one underlying quantitative trait locus (QTL) for milk production traits in the centromeric region of the bovine chromosome 14 [7, 8]. The signal transducers and activators of transcription (STATs), a family of transcription factors, mediate the actions of a variety of peptide hormones and cytokines [9]. STAT5, also known as mammary gland factor (MGF), was discovered initially as a PRL-induced transcription factor [10]. It is a key intracellular mediator of prolactin signalling and can activate transcription of milk protein genes in response to prolactin [10, 11]. 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 [12]. In cattle, the *STAT5A* and *STAT5B* genes were located close to each other (within 40 Kb) at chromosome 19 [13]. Antoniou et al. (1999) described two SSCP variants of the gene fragment that encodes the SH2 domain in bovine STAT5A protein [14]. Brym et al. (2004) detected a new SNP (A/G) located in the intron 9 of *STAT5A* gene at position 9501 [15]. The aim of this study was to investigate SNPs in *DGAT1* and *STAT5A* genes, and analyze the combined effect of *DGAT1* and *STAT5A* genes on milk production traits to provide the theoretical basis for goat breeding.

2. Materials and Methods

2.1. 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). They were reared in Qianyang county and Zhouzhi county of Shaanxi province, respectively. Health, fertility and milk recording was carried out by dairymen and veterinarians. Data was recorded in winter and spring parturitions of 2008 to 2011. Milk yields from first to 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 Company, Nova Zagora, Bulgaria). Five milliliters blood per goat were collected asepti-

cally from the jugular vein and kept in a tube containing anti-coagulant ACD (citric acid:sodium citrate:dextrose – 10: 27: 38). The genomic DNA was extracted from white blood cells using standard phenol-chloroform extraction protocol [16].

2.2. PCR Amplification

According to bovine DGAT1 and STAT5A genes (GenBank accession no. AJ318490 and AJ237937), fourteen pairs of primers were designed to amplify goat DGAT1 and STAT5A genes. Pairs of primer 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 2 × reaction mix (including 500 μM dNTP each; 20 mM Tris‐HCl; pH 9; 100 mM KCl; 3 mM MgCl2), 0.5 μM 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.

2.3. SNP Genotyping and Sequencing

The SSCP analysis of PCR products of primer pair 2 refers to An et al. (2011) [17]. In addition, PCR products (5μl) of primer pair 2 were mixed with 1 μl 10 × buffer, 3 U Eco81 Ⅰ (TaKaRa, Dalian, China) and 3.5 μl sterilized ddH2O, and then incubated for 1.5 h at 37°C. Digested products were subjected to PAGE (80 × 73 × 0.75 mm) in 1 × TBE buffer and constant voltage (110 V) for 1.5 h. After the polymorphisms were detected, amplicons representing unique banding patterns were sequenced in both directions in ABI 377 DNA analyzer (Applied Biosystems, Foster, California, USA) and the sequences were analyzed with DNAstar software (version 7.1) and Blast in NCBI (National Center for Biotechnology Information).

2.4. Statistical Analysis

The allelic frequencies, heterozygosity (He) and polymorphism information content (PIC) were calculated using Cluster-analysis software (version 1.2). 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 16 statistical software. The linear model applied was:

$$Y_{iknjlm} = \mu + G_i + B_k + P_n + N_j + (PG)_{ni} + S_l + E_{iknjlm} \quad \text{(model 1)}$$

where Y_{iknjlm} is the trait measured on each of the iknjlmth animal, μ is the overall population mean, G_i is the fixed effect associated with the ith genotype, B_k is the fixed effect associated with the kth breed, P_n is the fixed effect associated with the nth parity, N_j is the fixed effect associated with the jth number of kids born, $(PG)_{ni}$ is the interaction between the nth parity and ith genotype. S_l is the random effect associated with the lth sire, and E_{iknjlm} is the random error. The combined effects of *DGAT1* and *STAT5A* genes on milk production traits were analyzed with the following model:

$$Y_{iknjlm} = \mu + C_i + B_k + P_n + N_j + (PC)_{ni} + S_l + E_{iknjlm} \text{ (model 2)}$$

where Y_{iknjlm}, μ, B_k, P_n, N_j and S_l are the same as shown for model 1, C_i is the fixed effect associated with the ith combination genotype, and $(PC)_{ni}$ is the interaction between the nth parity and ith combination genotype.

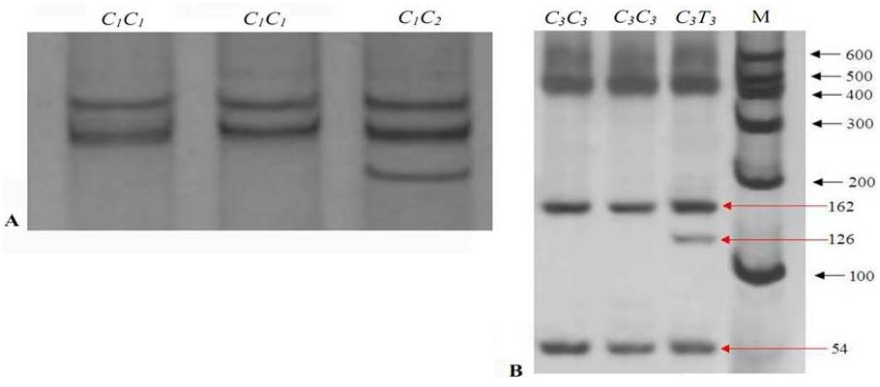
3. Results

3.1. SNPs Identification and Genotypes

The bands of different genotypes are shown in **Figure 1A** and **1B**. Comparisons among these nucleotide sequences of difference genotypes indicated that one base insertion (g.407_408insC, GenBank accession no. JF781126) was detected in the

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

Gene	Primer	Sequence (bp)	Ta (℃)	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



Note: Fragments including 36 bp of C_3T_3 genotype were invisible

Figure 1. SNP detection of PCR products at g.407_408ins*C* (A) and g.6852*C*>*T* (B) loci for two goat breeds.

intron 14 of DGAT1 gene (primer pair 1). Two base substitutions (g.6798C>T and g.6852C>T, GenBank no. JN091564) were detected in PCR products of primer pair 2 (exon 7), which were synonymous mutations. Because there is no homozygote at the g.6798 C>T locus, relevant data are not listed in Figure and Table. At g.407_408insC locus, C1C1 and C1C2 genotypes were found in SN and GZ breeds (**Figure 1A**). At g.6852C>T locus, C3C3 and C3T3 genotypes were detected in both breeds (**Figure 1B**). Allelic frequencies, He, and PIC are shown in **Table 2**. We found that the additive effect of DGAT1 and STAT5A SNPs on milk yield and fat percentage was extremely significant ($P < 0.001$), respectively. The additive effect between DGAT1 and STAT5A genes had extremely significant effects on milk fat percentage ($P < 0.001$) (**Table 3**).

3.2. Association and Effects of the SNPs and Combination Genotypes

In SN and GZ goat breeds, the genotypes of 528 individuals were analyzed for association with phenotypic data for milk yield and constituents at *g*.407_408ins*C* and *g*.6852*C>T* loci (**Table 4**). Milk protein and lactose did not show any significant association with genotypes. At *g*.407_408ins*C* locus, the does with C_1C_2 genotype had greater milk fat percentage than those with C_1C_1 genotype ($P < 0.05$). At *g*.6852*C>T* locus, the does with C_3T_3 genotype had greater milk yield than those with C_3C_3 genotype ($P < 0.05$) (**Table 4**). The does with $C_1C_1C_3T_3$ and $C_1C_2C_3T_3$ had higher milk yield than those with $C_1C_2C_3C_3$ ($P < 0.05$) (**Table 5**). In addition, the combined effect of $C_1C_2C_3T_3$ on milk fat percentage was the highest in comparison with other combination genotypes ($P < 0.05$).

4. Discussion

In this study, we analyzed the allelic frequencies of g.407_408insC and g.6852C>T in two goat breeds (n=528). The results showed that the C_2 (g.407_408insC locus) and T_3 (g.6852C>T) alleles had low frequencies (0.15-0.30), and C_2C_2

Table 2. Genotypic distributions, allelic frequencies of *g*.407_408ins*C* and *g*.6852*C>T* loci in two goat breeds.

Locus			Breed	
			SN	GZ
g.407_408ins*C*	Genotype	C_1C_1	197	141
		C_1C_2	88	102
	Allele	C_1	0.85	0.79
		C_2	0.15	0.21
	He		0.31	0.42
	PIC		0.23	0.28
g.6852*C>T*	Genotype	C_3C_3	112	106
		C_3T_3	173	137
	Allele	C_3	0.70	0.72
		T_3	0.30	0.28
	He		0.61	0.56
	PIC		0.33	0.32

Table 3. The additive effect of *g*.407_408ins*C* and *g*.6852*C>T* on milk yield (kg) and fat percentage (%).

Locus	Effect	Milk yield	Milk fat percentage
g.407_408ins*C*	Additivet	-1.58±4.93	0.15±0.03
	P value	0.75	<0.001
g.6852*C>T*	Additivet	18.78±10.08	0.03±0.06
	P value	<0.001	0.36
g.407_408ins*C* and g.6852*C>T*	Additivet × Additivet	-7.80±2.36	0.18±0.02
	P value	0.43	<0.001

Table 4. Association analysis of *g*.407_408ins*C* and *g*.6852*C>T* loci with milk yield (kg) and constituents (%) in goats (Xinong Saanen and Guanzhong goats).

Gene	Genotype	Milk yield (kg)	Milk fat (%)	Milk protein (%)	Lactose (%)
DGAT1	C_1C_1 (338)	653.71±2.25	3.38±0.03[a]	2.97±0.01	4.46±0.02
	C_1C_2 (190)	660.29±3.25	3.48±0.03[b]	2.96±0.01	4.45±0.02
STAT5A	C_3C_3 (218)	642.22±3.06[a]	3.41±0.03	2.97±0.01	4.47±0.02
	C_3T_3 (310)	665.67±2.48[b]	3.45±0.03	2.96±0.01	4.45±0.01

Note: The data are expressed as least square means ± standard errors. Values with different superscripts within the same column in particular population differ significantly at $P < 0.05$. Numbers in brackets indicate the number of samples. Milk samples from third lactation have been analyzed for milk constituents.

Table 5. Combined effects of *DGAT1* and *STAT5A* genes on milk yield (kg) and fat percentage (%) in goats (Xinong Saanen and Guanzhong goats).

Genotypic combination	Milk yield (kg)	Milk fat (%)	Milk protein (%)	Lactose (%)
$C_1C_1C_3C_3$ (147)	642.10±3.65[a]	3.44±0.03[a]	2.96±0.01	4.46±0.03
$C_1C_1C_3T_3$ (191)	664.35±3.32[b]	3.38±0.03[a]	2.95±0.01	4.42±0.02
$C_1C_2C_3C_3$ (70)	645.23±5.48	3.41±0.05[a]	2.97±0.02	4.45±0.04
$C_1C_2C_3T_3$ (120)	666.21±3.89[b]	3.59±0.04[b]	2.93±0.01	4.47±0.03

Note: The data are expressed as least square means ± standard errors. Values with different superscripts within the same column in particular generation differ significantly at $P < 0.05$. Numbers in brackets indicate the number of samples. Milk samples from third lactation have been analyzed for milk constituents.

(inset homozygote) and T_3T_3 (mutation homozygote) genotypes were not observed, respectively at the two loci in SN and GZ goat breeds. Flisikowski et al. (2003) reported C→T at position 6853 within the exon 7 of *STAT5A* gene and they found the *TT* genotype only in Polish native breeds (Polish Red and Polish White-Back cattle) [18]. We consider that the results can be explained by the following two reasons. (1) There is a lower frequency for missing genotypes, and the samples are small. (2) The missing genotypes of the two loci have negative effects on individual performance, so the individuals with missing genotypes have been eliminated in breeding process.

We firstly revealed the significant association of *DGAT1* indel (g.407_408insC) and *STAT5A* SNP (g.6852C>T) with milk yield and fat percentage in Chinese 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 can affect the mechanism of mRNA deadenylation and degradation [19-21]. Linkage disequilibrium with the causal mutation possibly affects the variation of milk production traits in goat [22]. Previous studies have demonstrated the importance of *DGAT1* and *STAT5A* genes in milk production traits in cattle [7,8, 23]. *DGAT1* candidate gene was found to have a significant effect not only on milk yield and component traits but also on the metabolism of intramuscular fat [7, 8, 24]. Amills et al. (2007) indicated T to C substitution at the intron 16 of goat *DGAT1* gene could be used as a marker in association studies with milk traits [25]. Dario et al. (2009) studied the effect of *STAT5A*/AvaI polymorphism on growth performance traits in Podolica bulls and suggested the superiority of C allele for growth performances because both *CC* and *CT* bulls tended to show a higher live weight and a faster growth in comparison with *TT* animals [11]. 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 [26]. Dario 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 protein content than those with *CT* genotype [27]. The biochemical and physiological functions, together with the results obtained in our study, indicate that the *DGAT1* and *STAT5A* genes might play important roles affecting milk production traits in goat. Genotypic value includes additive effect and dominant effect. Additive effect could be truly transmitted to offspring, so it is the focus of marker-assisted selection [28]. In this study, we took into account additive effect between SNP loci and milk production traits. The result showed the additive effect of g.407_408insC and g.6852C>T on milk yield and fat percentage was extremely 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 significantly affected milk yield and fat percentage. Kong et al. (2007) indicated no significant effects on economic traits in Hanwoo cattle were found in the separate analysis of K232A and T11993C polymorphisms of *DGAT1* gene, but the interaction between K232A and T11993C showed a significant effect ($P < 0.005$) on marbling score [24]. Based on the above considerations, we thought milk production traits were subjected to the impacts of g.407_408ins*C* and g.6852*C>T* loci, and there was an interaction between both loci.

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