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Effects of *CSN1S2* Genotypes on Economic Traits in Chinese Dairy Goats

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ABSTRACT: The aim of this study was to investigate allele frequencies at the *CSN1S2* locus in two Chinese dairy goat breeds and the effects of its variation on dairy goat economic traits. Seven hundred and eight goats from Xinong Saanen (XS, n = 268) and Guanzhong (GZ, N = 440) breeds were selected. The milk samples of 268 XS goats were collected during the middle of lactation, body size parameters (708 goats) and daily milk yield (202 goats) were registered. The RFLP (restriction fragment length polymorphism) and SSCP (single strand conformation polymorphism) were used to detect the polymorphisms in *CSN1S2*. The Hardy-Weinberg (HW) equilibrium and the associations between body size, milk yield and composition and the genotypes were calculated. The results revealed that only A and F *CSN1S2* alleles were found in the two Chinese dairy goat breeds. Allelic frequencies of A and F were 0.795, 0.205 and 0.739, 0.261 in Xinong Saanen and Guanzhong population respectively. Xinong Saanen breed was in Hardy-Weinberg equilibrium, while Guanzhong breed deviated from Hardy-Weinberg equilibrium ($p<0.05$). The association of polymorphism with economic traits indicated that the goats with FF genotype have higher milk fat and total solid concentration than those with AA and AF genotypes ($p<0.05$). (**Key Words:** *CSN1S2*, SNP, Dairy Goats, Milk, A and F Alleles)

INTRODUCTION

Casein genes are organized as a cluster, including in order α_{S1}-casein (*CSN1S1*), α_{S2}-casein (*CSN1S2*), β-casein (*CSN2*) and κ-casein (*CSN3*) (Ferretti et al., 1990; Threadgill and Womack, 1990). In goats, the entire casein cluster region spans about 250 kb on chromosome 6 (Hayes et al., 1993). In recent years, the genetic polymorphism of goat casein has raised considerable research interest because goat casein polymorphisms are related to milk quality, milk composition and technological properties (Martin et al., 2002).

The *CSN1S2* gene is 18.5 kb long and consists of 18 exons which vary from 21 to 266 bp (Groene et al., 1993). Seven alleles, showing three different synthesis levels, have been identified so far. The A, B, C, E and F alleles are characterized by point mutations related to single amino acid substitutions and associated with a normal amount of $\alpha\alpha_{S2}$-casein (2.5 g/L). The *CSN1S2* B allele, compared to the A allele, shows a G10A transition at exon 9, which causes a Glu64→Lys substitution in the mature protein, while the mutational events characterizing the C and E variants are substitution A5T (Lys167→Ile) and C83G (Pro193→Arg) at exon 16, respectively (Bouniol et al., 1994). The *CSN1S2* F allele is characterized by a G13C transition occurring at exon 3, which results in an amino acid substitution Val7→Ile (Bouniol et al., 1994; Veltri et al., 2000 Ramunno et al., 2001a). *CSN1S2* D is a rare defective allele characterized by a deletion, involving part of the exon 11 and of the following intron. The null allele *CSN1S2* O associated with a non-detectable amount of this casein fraction shows a G→A transition at the 80th nucleotide in exon 11, creating a premature stop codon at position 110 (Ramunno et al., 2001a).

China, with nearly four million goats, is one of largest producers of dairy goats in the world. Among which, the Xinong Saanen (SN) and Guanzhong (GZ) breeds are the best-known, not only due to their tolerance of crushed feed and the local harsh weather, but also to their high milk yield (Xu et al., 2003; Deng et al., 2010). While as for Chinese dairy goats, we found few reports about studying DNA polymorphism and their association with economic traits. The aim of the present study was to investigate the genetic structure of *CSN1S2* locus in these two important Chinese dairy goat breeds. Therefore, the genetic variations analysis

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Table 1. The primer pair sequences and their information of *CSN1S2 g*ene in goat

Loci	Sequences of primers	Annealing temperature	Amplified region/size bp	Allele
L1	R: 5'-TCTCTTGCCATCAAAACA-3' F: 5'-TGGTCTTTATTCCTCTCT-3'	50.4	Exon3 and flanking region/310 bp	F
L2	R:5'-GGACTCTAAATATACTTAATGAATT-3' F: 5'-GCTTATCGTCCACAGTAATCTT-3'	54.8	Exon9 and flanking region/261 bp	B
L3	R: 5'-GACACATAGAGAAGATTC-3' F: 5'-CGTTGGGACATTTTATCT-3'	50.6	Exon11 and partial intron11/301 bp	O, D
L4	R: 5'-GGTTAGGTCTAGGTGTTCTGA-3' F: 5'-TTTTTATTACAAAAGACAACT-3'	50.4	Exon16 and flank region/226 bp	C, E

Numbering of L2 and L4 loci primers agrees with the nucleotide sequence of goat *CSN1S2* gene (GenBank No. AJ297310 and AJ297315).

of the *CSN1S2* gene in 708 Chinese dairy goats may provide useful information related to the understanding of their genetic characteristics. The genetic associations related to goat milk yield and composition in Chinese dairy goat breeds will possibly contribute to improving the quality of dairy goat breeds in China.

MATERIALS AND METHODS

Samples collection and DNA extraction

Blood samples of 708 goats were collected from two Chinese dairy goat breeds of Xinong Saanen (XS) and Guanzhong breed (GZ) as follows: Qianyang Dairy Goat Breeding Farm (XS breed, n = 202), Northwest A&F University Goat Farm (XS breed, n = 66) and Shaanxi Dairy Goat Breeding Centre (GZ breed, n = 440) in Sanyuan county, Shaanxi province of China. The genomic DNA was extracted from blood by a standard phenol-chloroform method (Chomczynski and Sacchi, 1987).

Milk samples and data collection

The milk yield of second lactation of the XS breed (n = 202) in Qianyang Dairy Goat Breeding Farm was recorded. In addition, a 50 ml sample of morning milk obtained from goats of the XS breed in Northwest A&F University Goat Farm and Qianyang Dairy Goat Breeding Farm was collected (n = 268). The milk samples were stored at 4°C until the next day when they could be analyzed by MilkoScan FT120 (FOSS Corporation, Denmark) for concentrations of milk fat, protein, lactose, total solids (TS), milk solids-not-fat (SNF) and density of milk. Body size parameters of all goats are documented, including Withers height, Body length and Chest circumference.

Genotyping of *CSN1S2* locus

CSN1S2 F, O and D Alleles were detected by means of RFLP-PCR (restricted fragment length polymorphism-PCR) according to Ramunno et al. (2001a). Furthermore, based on the nucleotide sequence characterization of *CSN1S2* B, C and E alleles, the PCR-SSCP procedure was developed for genotyping them by analyzing polymorphisms of exon 9 and exon 16 of *CSN1S2* gene (Table 1). The PCR-SSCP and RFLP-PCR procedures were carried out based on Yue et al. (2011) and Ramunno et al. (2001a) respectively.

Statistical analysis

Genotypic and allelic frequencies, as well as Hardy–Weinberg equilibriums, were directly calculated. Mixed model analyses for milk yield, milk composite and body size parameters were performed using the general linear models procedure of SSPS 17.0 (SPSS Inc., Chicago, IL, USA). The model for dairy goats included marker genotype, year, kidding season (spring versus fall) and lactation number as fixed effects, the linear and quadratic effects of milk yield, milk composite and body size parameters included as covariables and doe as a random effect. The factor, number of kids, had not been included in the statistical model for following reason. The ewes in this study predominantly had one kid, but does that produced twins were also part of the dataset. Data were presented as least squares means with associated standard error.

RESULTS AND DISUSSION

The *CSN1S2* locus is characterized by the presence of seven alleles, of which, A, B, C, E and F are associated with normal α_{s2}-casein level (2.5 g/L). D allele is associated with reduced αs_2-casein content, and O allele is associated with a non-detectable amount of this casein in milk (Ramunno et al., 2001a, b). Interestingly, in present study only *CSN1S2* F and A alleles were detected (Figure 1), while B, C, E, D and O alleles were absent. Overall, three different genotypes, AA, AF and FF were identified in the two Chinese dairy goat breeds. Allele and genotype frequencies are shown in the Table 2. Frequencies of A and F alleles in XS and GZ breeds are 0.795/0.205 and 0.739/0.261, respectively. XS breed was found to follow the Hardy-Weinberg equilibrium, whereas GZ breed was deviated from Hardy-Weinberg equilibrium ($p<0.05$). Associations of second lactation milk yield, body size and milk composition and genotype were calculated by SPSS 17.0 (Table 3). Statistical analysis

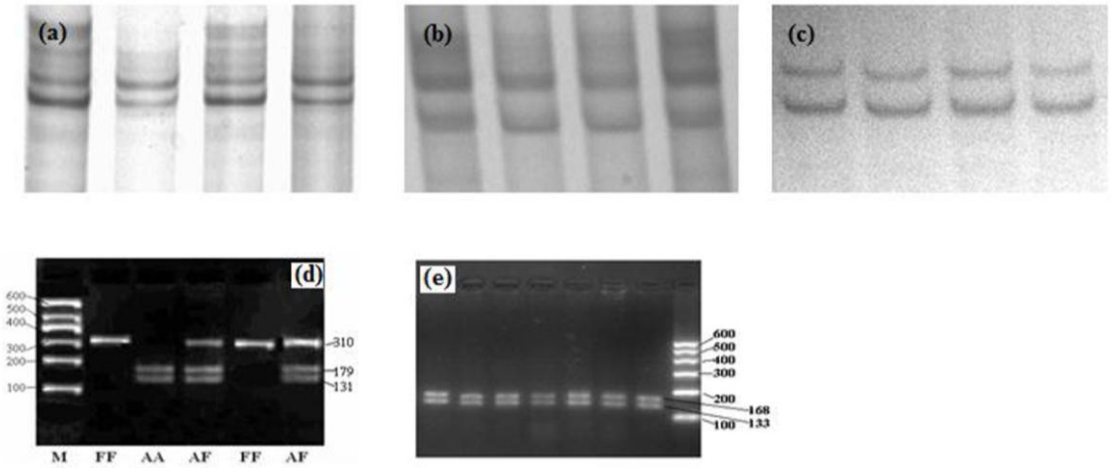

Figure 1. PCR products of five loci analyzed by PCR-SSCP and PCR-RFLP. Figure 1-a, b, c are the PAGE-electrophoresis patterns of PCR-SSCP for L1 (a), L3 (b), L5 (c) locus of *CSN1S2* gene, respectively. They all show monopolymorphy. Figure 1-d is the 2.5% agarose gel electrophoresis for *Alw26* I digesting PCR products of *CSN1S2* L2 locus. Figure 1-e is the 2.5% agarose gel electrophoresis for *Nco* I digesting PCR products of *CSN1S2* L4 locus.

indicated that the individuals with FF genotype have higher milk fat and total solid than those of AA and AF genotypes. However, this mutation has no influence on milk yield and body size parameters based on the current study (Table 3). Previous study revealed that dairy goats with *CSN1S2* FF genotype had a significantly lower milk yield than other individuals, whereas this study did not show that goats carrying FF genotype had a significantly lower milk yield (Lan et al., 2005). This can be explained by the sample size of the previous study being too small (69 individuals).

It is interesting that only two *CSN1S2* alleles (A and F allele) were detected in two Chinese dairy goat breeds in the present study, which is greatly different from the *CSN1S2* allelic distribution in European dairy goat breeds. The frequency of *CSN1S2* A, B and C alleles were estimated to be 0.85, 0.04 and 0.11 in French Alpine and Saanen breeds

Table 2. Genotype and allelic frequencies of *CSN1S2* gene in 2 dairy goat breeds

Breeds	Genotypes			Samples	Genotype frequencies			Allele frequencies		x^2
	AA	AF	FF	N	P_{AA}	P_{AF}	P_{FF}	A	F	
XS	170	86	12	268	0.634	0.321	0.045	0.795	0.205	0.071
GZ	250	150	40	440	0.568	0.341	0.091	0.739	0.261	6.029*

Value with * * and * differ significantly at p<0.01 and p<0.05 respectively. 2. $x^2_{0.05}$ (df = 1) = 3.84, $x^2_{0.01}$(df = 1) = 6.63.

Table 3. Associations of polymorphism at *CSN1S2* gene L2 locus with economic traits in dairy goats

Traits	Genotypes		
	AA	AF	FF
Fat (%)	2.71±0.12[a]	2.69±0.19[a]	3.86±0.42[b]
Protein (%)	3.25±0.05	3.20±0.08	3.43±0.18
TS (%)	11.29±0.15[a]	11.18±0.23[a]	12.57±0.52[b]
SNF (%)	8.48±0.06	8.38±0.23	8.61±0.20
Lactose (%)	4.12±0.03	4.06±0.47	4.04±0.10
Density (g/L)	1,030.84±0.23	1,030.26±0.38	1029.69±0.78
Milk yield (kg)	860.90±33.20	825.54±13.43	822.79±8.48
Withers height (cm)	70.99±0.35	71.27±0.41	71.42±0.79
Body length (cm)	80.03±0.44	80.18±0.69	79.66±1.18
Chest circumference (cm)	91.98±2.62	94.05±2.98	93.68±5.17

Values in the same row labeled with the different letter (a, b) are significantly different.
The number of goats carrying AA, AF and FF genotypes with body size parameters (Withers height, Body length and Chest circumference) represent 420, 236 and 52 individuals, respectively.
In milk composite traits (Fat, Protein, TS, SNF, Lactose, and Density), AA, AF and FF genotypes represent 170, 86 and 12 individuals, respectively.
In the milk yield, AA, AF and FF genotypes represent 139, 54 and 9 individuals, respectively.

before discovering the E, D, F and O alleles (Bouniol et al., 1994). While C and F alleles are predominant in local Italian goat breeds (Sacchi et al., 2005). The relatively high incidence of O allele (0.146) appeared in Hungarian Milking Goats (Kusza et al., 2007). There needs to be a larger number of Chinese dairy goats and more goat breeds sampled to confirm whether *CSN1S2* alleles in addition to A and F exist in Chinese goat breeds.

A previous study (Ramunno et al., 2001a) reported that *CSN1S2* homozygous genotypes were associated with good quality of milk proteins. We also detected this tendency, but this difference was not significant ($p>0.05$), maybe due to the small sample size. This study indicated that F allele was associated with good quality of milk fat. According to this finding, the selection of goats with higher milk fat should aim to increase the homozygous *CSN1S2* FF genotype.

The defective D and O alleles were not identified in this study. Both alleles showed a very low or null frequency in previous studies (Sacchi et al., 2005; Marletta et al., 2005; Kusza et al., 2007). The results of the trial allow us to presume that the *CSN1S2* genetic polymorphism in the two Chinese breed does not affect the a_{s2}-casein quantity. *CSN1S2* locus is closely linked to *CSN1S1*, *CSN2* and *CSN3* loci and alleles of these loci are inherited together as allele groups called haplotype (Hayes et al., 1993; Rijnkels, 2002). It means that polymorphisms occurring at a casein locus have to be considered in the context of the casein gene cluster, using information deriving from the entire casein haplotype (Hayes et al., 2006; Finocchiaro et al., 2008). The goats analyzed in this study showed the occurrence of defective alleles at the *CSN1S1* locus, coding for αs_1-casein, such as the F, 01 and N alleles in our previous studies (Yue et al., 2011). Therefore further studies are required to determine the relationship between the absence of *CSN1S2* D and *CSN1S2* O alleles and the distribution of alleles at *CSN1S1*, *CSN2* and *CSN3* loci in the populations studied.

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REFERENCES

Bouniol, C., G. Brignon, M. F. Mahé, and C. Printz. 1994. Biochemical and genetic analysis of variant C of caprine αs_2 -casein (Capra hircus). Anim. Genet. 25:173-177.

Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162:156-159.

Deng, C. J., R. N. Ma, X. P Yue, X. Y. Lan, H. Chen, and C. Z. Lei. 2010. Association of IGF-I gene polymorphisms with milk yield and body size in Chinese dairy goats. Genet. Mol. Biol. 33:266-270.

Ferretti, L., P. Leone, and V. Sgaramella. 1990. Long range restriction analysis of the bovine casein genes. Nucleic Acids Res. 18:6829-6833.

Finocchiaro, R., B. J. Hayes, M. Siwek, R. J. Spelman, J. B. C. H. M. van Kaam, T. Å dnøy, and B. Portolano. 2008. Comparison of casein haplotypes between two geographically distant European dairy goat breeds. J. Anim. Breed. Genet. 125:68-72.

Groenen, M. A., R. J. M. Dijkhof, A. J. M. Verstege, and J. J. Van der Poel. 1993. The complete sequence of the gene encoding bovine αs_2-casein. Gene 123:187-193.

Hayes, B., N. Hagesæther, T. Å dnøy, G. Pellerud, P. R. Berg, and S. Lien. 2006. Effects on production traits of haplotypes among casein genes in Norwegian goats and evidence for a site of preferential recombination. Genetics 174:455-464.

Hayes, H., E. Petit, C. Bouniol, and P. Popescu. 1993. Localization of the alpha S2-casein gene (CASAS2) to the homologous cattle, sheep and goat chromosomes 4 by *in situ* hybridization. Cytogenet. Cell Genet. 64:281-285.

Kusza, S., G. Veress, S. Kukovics, A. Jávor, A. Sanchez, A. Angiolillo, and A. Bősze. 2007. Genetic polymorphism of α_{s1} and α_{s2}-caseins in Hungarian milking goats. Small Rumin. Res. 68:329-332.

Lan, X. Y., H. Chen, R. F. Zhang, Y. Tian, Y. D. Zhang X. T. Fang, W. B. Sun, C. Z. Lei, and S. R. Hu. 2005. Association of polymorphisms of *CSN1S2* gene with average milk yield and body sizes indexes in Xinong Saanen dairy goat. Acta Veterinaria Et Zootechnica Sinica 36:318-322 (in Chinese).

Marletta, D., S. Bordonaro, A. M. Guastella, A. Criscione, and G. D'Urso. 2005. Genetic polymorphism of the calcium sensitive casein in Sicilian Girgentana and Argentata dell'Etna goat breeds. Small Rumin. Res. 57:133-139.

Martin, P., M. Szymanowska, L. Zwierzchowski, and C. Leroux. 2002. The impact of genetic polymorphisms on the protein composition of ruminant's milks. Reprod. Nutr. Dev. 42:433-459.

Ramunno, L., G. Cosenza, M. Pappalardo, E. Longobardi, D. Gallo, N. Pastore, P. Di Gregorio, and A. Rando. 2001a. Characterization of two new alleles at the goat *CSN1S2* locus. Anim. Genet. 32:264-268.

Ramunno, L., E. Longobardi, M. Pappalardo, A. Rando, P. Di Gregorio, G. Cosenza, P. Mariani, N. Pastore, and P. Masina. 2001b. An allele associated with a non-detectable amount of αs_2-casein in goat milk. Anim. Genet. 32:19-26.

Rijnkels, M. 2002. Multispecies comparison of the casein gene loci and evolution of casein gene family. J. Mammary Gland Biol. Neoplasia 7:327-345.

Sacchi, P., S. Chessa, E. Budelli, P. Bolla, G. Ceriotti, D. Soglia, R. Rasero, E. Cauvin, and A. Caroli, 2005. Casein haplotype structure in five Italian goat breeds. J. Dairy Sci. 88:1561-1568.

Threadgill, D. W., and J. E. Womack. 1990. Genomic analysis of the major bovine milk proteins genes. Nucleic Acids Res. 18: 6935-6942.

Veltri, C., R. Lagonigro, E. Pietrolà, M. D'Andrea, F. Pilla, and L. Chianese. 2000. Molecular characterization of the goat α_{s2} -casein E allele and its detection in goat breeds of Italy, in:

Proceedings 7th International Conference on Goats, Tour, France, 15-18 May, p. 727.

Xu, G. Y., J. B. Pu, and T. Y. Chen. 2003. On development status and countermeasures of goat industry in western China. China Herbivores Suppl. 1:31-32.

Yue, X. P., X. M. Zhang, W. Wang, R. N. Ma, C. J. Deng, X. Y. Lan, H. Chen, F. Li, X. R. Xu, Y. Ma, and C. Z. Lei. 2011. The *CSN1S1* N and F alleles identified by PCR-SSCP and their associations with milk yield and composition in Chinese dairy goats. Mol. Biol. Rep. 38:2821-2825.