Effects Of Genetic Variability Of The Goat *Weaver* Gene On Milk Traits

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Introduction

The *weaver* gene with three coding exons located on human chromosome 21 (21q22.1-22.2) encodes for the GIRK2 protein subunits of a human ATP-sensitive K-channel which plays a central role in insulin release from pancreatic β-cells (Sakura, H., Bond, C., Warren-Perry, M. et al. (1995)) and is widely and distinctively expressed in the central nervous system (CNS) (Ikeda, K., Yoshii, M., Sora, I. et al (2003)). The mice phenotype *weaver* mutation (Gly-Ser), characterized by ataxia, tremor, male infertility, tonic-clonic seizures and the degeneration of cerebellum granule neurons (Yao, W., Zhong, J., Yu, T. et al. (2008)), was caused by a point missense mutation (Gly-Ser) in the H5 region of *weaver* gene (Patil, N., Cox, D., Bhat, D. et al. (1995)). Therefore, *weaver* gene participates in a wide range of physiologic responses (Benarroch, E. (2009)).

Cattle weaver syndrome is a recessive genetic disease which was found in 36 purebred Brown Swiss cattle in 1973 (Stuart, L., and Leipold, H. (1985)). Interestingly, cattle weaver syndrome is related to cattle milk production (Georges, M., Dietz, A., Mishra, A. et al. (1993)). Weaver carrier cows have an higher producing milk than noncarrier ones (Hoeschele, I., and Meinert, T. (1990)). Thus, *weaver* gene is an important potential candidate gene for production performance in livestock.

Therefore, the aim of present study was to scan SNPs within the caprine *weaver* gene by using PCR-SSCP, DNA sequencing and Forced-PCR-RFLP methods and to determine the associations of the polymorphisms with milk yield and milk composition.

Materials and methods

DNA samples. Genomic DNA samples were obtained from 708 healthy individuals belonging to 2 indigenous Chinese goat breeds, namely, XNSN, dairy breed, n = 268; GZ,

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dairy breed, n = 440. Records of total milk yield as well as fat, protein, solids-not-fat, lactose and total solids percentage during a whole lactation, which were collected form 268 XNSN dairy goats with 2yr-3yr were used for statistical analysis.

PCR Conditions and detecting novel SNP. Based on the bovine sequence (GenBank accession No. NC_007299), 8 pair of primers to amplify different PCR products (P1-P8) of the carpine *weaver* gene were designed (data not shown). PCR products were analyzed for single-strand conformation polymorphisms (SSCP) according to the description from Lan, X., Pan, C., and Chen, H. (2007). Based on the findings of SSCP, the putative polymorphic DNA samples were sequenced with the forward and reverse directions in ABI PRISM 3730 DNA analyzer and sequences were analyzed with BioXM software.

Statistical analysis. Mixed model analyses for milk traits were performed using the SAS Mixed procedure (SAS, 1999). The model for XNSN included marker genotype, sire, lambing season, age and lactation number as fixed effects, the linear and quadratic effects of lactation length as covariates and doe as a random effect. The factor number of kids was not included in the statistical model. Because XNSN does predominantly had one kid, but does that lambed twins were also part of the dataset. Data are presented as least squares means with associated standard error.

Results and discussions

SNPs and PCR-RFLP analysis. We preliminarily carried out 8 pair of primers sequences to detect the polymorphisms of caprine *weaver* gene by PCR-SSCP. However, only exon 4 and flanking 3'UTR region, showed polymorphism. DNA from 8 randomly chosen goats with different SSCP patterns were sequenced in both directions and some of their sequences were deposited in the GenBank database (Accession No. FJ794608, FJ823054-FJ823056 and FJ973156-FJ973159). High homology was observed with the bovine *weaver* gene (GenBank accession No. NC_007299). Sequencing and alignments comparisons showed four novel SNPs: G5896A and T6256A in the exon 4, C99045T and C99116T in the flanking 3'UTR region of *weaver* gene.

Allele substitution effect. We reported that one SNP locating on exon 4 was significantly associated with milk yield (P = 0.045) (Table 1). However, no significant difference was found in milk composition. The possible explanations are shown in the following. Firstly, this is accordance with the function of the *weaver* gene which plays an essential role in the function of many organs, such as endocrine organ, and may be involved in the regulation of hormone secretion (Benarroch, E. (2009)). Secondly, T6256A-A is possibly linked to another

mutation in the coding or regulatory regions of the gene which is a causal mutation for the milk traits (Stachowiak, M., Szydlowski, M., and Cieslak, J. (2007)), but not G5896A-A. Moreover, although the function of synonymous mutation remains unknown, it is generally accepted that synonymous mutation plays an important role in regulating gene expression. The SNPs in the coding regions may be useful to measure allelic imbalances at the mRNA level, at least some synonymous changes can affect splicing, mRNA stability or translational efficiency (Reincke, S., Govbakh, L., Wilhelm B. et al. (2008)).

Table 1: Associations of different genotypes within the *weaver* gene with milk yield and milk composition in Xinong Sannen dairy goat (Mean±S.E.)

Loci	GE	First MY	Second MY	Fat	Protein	SNF	TS
T6256A	TT	609.81±12.37	822.24±21.34^{b}	2.63±0.27	3.19±0.11	8.33±0.12	11.07±0.33
	TA	612.72±7.43	836.69±12.32^{b}	2.89±0.14	3.27±0.06	8.50±0.07	11.48±0.18
	AA	629.43±11.03	879.03±15.43^{a}	2.69±0.20	3.18±0.09	8.41±0.09	11.20±0.25
	P	*P*=0.384	*P*=0.045*	*P*=0.593	*P*=0.686	*P*=0.465	*P*=0.456
C99045T	CC	590.47±9.66^{b}	827.16±18.79	2.65±0.16	3.11±0.07^{b}	8.33±0.08^{b}	11.44±0.20
	CT	624.39±8.14^{a}	841.73±13.24	2.68±0.15	3.21±0.07^{b}	8.60±0.07^{a}	11.40±0.16
	TT	628.17±13.66^{a}	847.03±16.17	2.87±0.20	3.52±0.10^{a}	8.76±0.09^{a}	11.63±0.30
	P	*P*=0.015*	*P*=0.087	*P*=0.696	*P*=0.027*	*P*=0.032*	*P*=0.264

Note: GE=genotype; MY = milk yield; SNF = solids-not-fat; TS = total solids; S.E. = standard error of the mean. Values with different superscripts within the same line differ significantly $P<0.05$ (a, b).

In this study, we revealed the association of the polymorphisms of *weaver* gene with milk yield, protein and solids-not-fat percentage in the XNSN. Significant statistical results were found in milk yield among different genotypes in C99045T polymorphism locus ($P = 0.015 < 0.05$). Protein and solids-not-fat content were higher in milk from C99045T-TT compared with C99045T-CC genotypes (3.52 vs. 3.11% and 8.76 vs. 6.33%, respectively). Moreover, no significant relationships were observed between the DNA polymorphisms and growth traits (body height, body length and chest circumference) ($P > 0.05$, data not shown). In dairy cattle, the influence of *weaver* on milk production traits has already been described (Georges, M., Dietz, A., Mishra, A. et al. (1993)). C99045T polymorphism locus showed the stronger association with milk traits in the XNSN breed. However, no influence of *weaver* gene variant on milk traits was detected in the C99116T polymorphism locus ($P > 0.05$, data not shown). We also estimated the effect of the combination of the 2 SNPs. But, no significant difference was found in milk traits. We consider that these associations can be explained by the following possible reasons. Although the mutation of 3'UTR doesn't change

amino acid sequence, it regulates the expression of *weaver* gene. It has been observed that the sequences in the 3'UTR can affect mRNA deadenylation and degradation and significantly associated with mRNA stability (Kamiyama, M., Kobayashi, M., Araki, S. et al. (2007)). T (C99045T-T) in the 3′UTR may be the functional nucleotide that increases *weaver* expression. Moreover, linkage disequilibrium with the causal mutation possibly affects variation in milk traits. If C99045T-T is in linkage disequilibrium with a gene affecting variation in milk traits, segregation based on marker alleles would result in phenotypic differences (Ester, W., Van-Meurs, J., Arends, N. et al. (2009)). Hence the C99045T-T allele may be beneficial for improving milk yield. Therefore, NC_007299:g.99045C > T mutation may directly or indirectly influence the stability of the mRNA of *weaver* gene, and consequently, the amount of protein produced, which needs further study.

Conclusions

In conclusion, in this study, we reported the 4 polymorphisms in goat *weaver* gene, and their associations with the production traits and milk composition in goat. Some of those with better performance of C99045T-TT and T6256A-AA genotypes could be used for the breeding of new breeds of dairy goat in China. Furthermore, this study contributed to evaluating it as genetic marker in goat breeding, genetics and weaver syndrome.

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