Full Length Research Paper

Weaver gene 3'UTR novel mutations: Associations with production traits and milk composition in dairy goat

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Accepted 19 September, 2011

Our recent report on a parallel increase in the milk yield of weaver gene mutation suggests that weaver gene is a candidate marker for quantitative traits in farm animals with respect to milk production traits. To further understand the effects of weaver gene variant on fat, protein, solids-not-fat, lactose and total solids percentage, two novel single nucleotide polymorphisms (SNPs) located on the flanking 3'UTR region were investigated by TaqI polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and HhaI forced PCR-RFLP in a sample of 1,019 individuals from four Chinese indigenous goat breeds. In the TaqI and HhaI analyses, the frequencies of allele T2 and H2 in Xinong Sannen dairy (XNSN dairy) and Guanzhong dairy (GZ dairy) populations were significantly greater than those of Shanbei White Cashmere (SBWC) and Xinjiang White Cashmere (XJWC) goats, respectively. Relationships analyses between the two SNPs and milk performance traits and growth traits were performed. T2T2 animals had significantly greater milk yield, protein and solids-not-fat percentage than those with the T1T1 genotype. Thus, weaver/TaqI polymorphism appears to be a promising marker to improve milk production traits in goat.

Key words: Dairy goat, weaver gene, milk performance traits, single nucleotide polymorphism.

INTRODUCTION

Many important genes have been discovered to be involved in controlling milk traits in livestock. And it is important to realize that milk traits are controlled by polygenes with pleiotropic effect (Moioli et al., 2007; Dagnachew et al., 2011; Glantz et al., 2011). However, the major gene model suggests that a few genes may account for relatively large proportion of the genetic variation. The weaver gene (also named as KCNJ6) encodes for the GIRK2 protein subunits of a human ATP-sensitive K+-channel that is widely and distinctively expressed in the central nervous system (Ikeda et al., 2003). Two silent mutations (rs702859 and rs2070995) with similar frequencies in normal and non-insulin-dependent diabetic patients were identified (Sakura et al., 1995). One nonsense mutation (rs59497335) has been listed in the NCBI dbSNP database. The mice phenotype weaver mutation (Gly-Ser) in the H5 region caused ataxia, tremor, male infertility, tonic-clonic seizures and the degeneration of cerebellum granule neurons (Goldowitz, 1989; Patil et al., 1995). Moreover the GIRK1/GIRK2 gene may be involved in the inhibitory regulation of the release of oxytocin and vasopressin from the supraoptic nucleus in rat (Li et al., 2001). Therefore, weaver gene participates in a wide range of physiologic responses (Alexander et al., 2008; Benarroch, 2009).

The weaver gene with 5 exons located on cattle chromosome 1 (NC_007299) encodes for the GIRK2 protein. In 1973, cattle weaver syndrome was a cattle progressive degenerative myeloencephalopathy and recessive genetic disease which was found in 36 purebred Brown Swiss cattle (Stuart and Leipold, 1985; Tenhumberg et al., 1994). Interestingly, weaver carriers produce more milk than noncarriers (Hoeschele and Meinert, 1990).
Cattle weaver syndrome is related to cattle milk production (Georges et al., 1993). Seven single nucleotide polymorphisms (SNPs) (rs41706488, rs41706492 and rs41615623) have been reported in the bovine weaver gene in GenBank (http://www.ncbi.nlm.nih.gov). Moreover, genetic characteristics of seven microsatellite loci linked with weaver gene on BTA1 revealed that BM6438, BMS711, BMS2321 and TGLA116 were potential DNA markers of milk production (Shan et al., 2002). Thus, weaver gene is an important potential candidate gene for the production performance in livestock.

Therefore, the aim of this study was to scan SNPs within the caprine weaver gene by using DNA sequencing and PCR-RFLP methods and to determine the associations of the polymorphisms between milk production traits and milk composition.

MATERIALS AND METHODS

Animal data

DNA was extracted from blood (white blood cell) collected from 1,019 healthy and unrelated individuals belonging to our indigenous Chinese goat breeds, namely, Xinong Sannen dairy (XNSN, dairy breed, n = 268), Guanzhong dairy (GZ, dairy breed, n = 440), Xinjiang White Cashmere (XJWC, wool breed, n = 119), and Shanbei White Cashmere (SWC, wool breed, n = 192), which were from Shaanxi, Shaanxi, Xinjiang Uygur Autonomous Region and Shaanxi province, respectively. Body height, length and chest circumference were measured on 268 XNSN dairy goats two to three years, which were collected from 268 XNSN dairy goats two to three years, were used for statistical analysis. Milk compositions were measured by MilkoScan FT120 following the instruction. There were no milk yield records of GZ dairy goats, because the farm of GZ dairy goats was a new farm.

PCR conditions

Based on the bovine sequence (GenBank accession No. NC_007299), four pair of primers for the amplification of different fragments of the caprine weaver gene (P1-P4) were designed (Table 1). The 25 μl volume contained: 50 ng genomic DNA, 0.5 μM of each primer, 1 μl buffer (including 1.5 mM MgCl2), 200 μM dNTPs and 0.625 units of Tag DNA polymerase (MBI, Vilnius, Lithuania). The cycling protocol was 5 min at 95°C, 35 cycles of 94°C for 30 s, annealing for 30 s, 72°C for 40 s, with a final extension at 72°C for 10 min. The expected sizes of the amplifications, which are shown in Table 1, were confirmed by 1.0% agarose gels electrophoresis stained with ethidium bromide.

Detecting novel polymorphisms within goat weaver gene by PCR-SSCP and DNA sequencing

PCR products were analyzed for single-strand conformation polymorphisms (SSCP) in four loci. Aliquots of 5 μl PCR products from 80 randomly selected individuals for each breed were mixed with 5 μl denaturing solution (95% formamide, 25 mM EDTA, 0.025% Xylene-Cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C and chilled on the ice. Denatured DNA was subjected to PAGE (80 x 73 x 0.75 mm) in 1 x TBE buffer and constant voltage (200 V) for 2.5 to 3.0 h at 4°C. The gels were stained with 0.1% silver nitrate (Lan et al., 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 (Version 2.6).

Genotyping of TaqI and HhaI weaver allele by PCR-RFLP

The 20 µl 173 bp PCR products of P4 locus were digested with 10 U TaqI (MBI, Vilnius, Lithuania) for 5 h at 65°C and HhaI (MBI, Vilnius, Lithuania) for 5 h at 37°C following the supplier’s protocol, respectively. The digested products were analyzed by electrophoresis for 2 h in 3.0% agarose gels stained with ethidium bromide.

Statistical analysis

Estimate of linkage disequilibrium was performed by SHEsis software (Li et al., 2009). Genotypic and allelic frequencies and Hardy-Weinberg equilibrium were directly calculated according to the TaqI, HhaI PCR-RFLP analysis of the goat weaver P4 locus. Homozygosity was calculated by Yeh’s method using PopGene software (Version 1.3.1) (Yeh et al., 1999). Differences for these frequencies among/between different populations were analyzed using the chi-square test, which was performed by SPSS software (Version 16.0).

A full animal model and then a reduced animal model were used in a joint analysis of the growth traits and genotypes according to the description from Lan et al. (2007). 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; and rs41615623) have been reported in the bovine weaver gene in GenBank (http://www.ncbi.nlm.nih.gov). Moreover, genetic characteristics of seven microsatellite loci linked with weaver gene on BTA1 revealed that BM6438, BMS711, BMS2321 and TGLA116 were potential DNA markers of milk production (Shan et al., 2002). Thus, weaver gene is an important potential candidate gene for the production performance in livestock.

Therefore, the aim of this study was to scan SNPs within the caprine weaver gene by using DNA sequencing and PCR-RFLP methods and to determine the associations of the polymorphisms between milk production traits and milk composition.

RESULTS AND DISCUSSION

Based on the PCR-SSCP, flanking 3’UTR region showed polymorphism (Figure 1a). DNA from eight randomly chosen goats with different SSCP patterns were sequenced in both directions and some of their identities was identical with the bovine weaver gene (GenBank accession No. NC_007299) after the
Table 1. Primers information of goat weaver gene.

<table>
<thead>
<tr>
<th>Loci</th>
<th>Primer (5'-3')</th>
<th>Fragment (bp)</th>
<th>Tm (°C)</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1F: CAGGTGTATGACTGGCGAGTCTG 1R: TGGGCACGAATGCAAATAC</td>
<td>319</td>
<td>58.5</td>
<td>Exon 1 and part of intron 1 (18-336)*</td>
</tr>
<tr>
<td>P2</td>
<td>2F: CTACTCAGGCTGCCCTTAACA 2R: CCATCAGGACAAGGCTCAC</td>
<td>515</td>
<td>62.0</td>
<td>Part of intron 2, exon 3 and part of intron 3 (4812-5326)</td>
</tr>
<tr>
<td>P3</td>
<td>3F: GCATCCAAGAGTTTCCTTG 3R: TCAGAACAGAAATGCCTACT</td>
<td>376</td>
<td>55.0</td>
<td>Part of 3' flanking (98970-99345)</td>
</tr>
<tr>
<td>P4</td>
<td>4F: GCATCCAAGAGTTTCCTTG 4R: CTTCACCTCACCTGGTCTGTAAGC</td>
<td>173</td>
<td>56.0</td>
<td>Part of 3' flanking (98970-99142)</td>
</tr>
</tbody>
</table>

The reverse primer of P4 was designed to genotype the two mutations which were detected by SSCP in P3. The underlined base shows the incorporated mismatch creating a restriction site. * Ref. NC_007299.

Figure 1. Electrophoretic patterns of PCR-SSCP and PCR-RFLP of goat weaver gene. 1a, PCR-SSCP of goat weaver gene; Lane 1, 3, C-C/C-C); lane 2, C-C/T-C; lane 2, C-T/C-C) and lane 5, (T-T/C-T); 1b, the TaqI PCR-RFLP of goat weaver gene; lane 1, T1T2 genotype; lane 2, 3 and 5, T1T1 genotype; lane 4, T1T1 genotype; 1c the HhaI forced PCR-RFLP of goat weaver gene; lane 1, H1H2 genotype; lane 2 to3, H1H1 genotype; lane 4 to 5; H1H2 genotype; M, (400, 300, 200, and 100 bp).

comparison among the goat sequences following the SSCP two novel SNPs: NC_007299:g.99045C > T; NC_007299:g.99116C > T in the flanking 3'UTR region of weaver gene. Interestingly, the NC_007299:g.99045C > T mutation abolished a TaqI endonuclease restriction site (TCGA). In order to exactly detect the NC_007299:g.99116C > T mutation, the forced PCR-RFLP method was used to detect this mutation (Cox, 2006). The reverse primer (Table 1-P4R) was redesigned. Therefore, the 173 bp amplification contains two endonuclease restriction recognition sites for the detection of the mutations in weaver gene. At the TaqI locus, digestion of the 173 bp PCR fragment with TaqI resulted in fragment lengths of 98 and 75 bp bands for genotype T1T1 individual (homozygous); T1T2 (heterozygous) showed 173, 98 and 75 bp bands; T2T2 (homozygous) showed 173 bp (Figure 1b). At the HhaI locus, digestion of the 173 bp PCR fragment with HhaI resulted in fragment lengths of 148 and 25 bp bands for genotype H1H1 individual (homozygous); H1H2 (heterozygous) showed 173, 148, and 25 bp bands; H2H2 (homozygous) showed 173 bp (Figure 1c). It was noted that the 25 bp fragment was too short to be visible in Figure 1c.

The two novel SNPs of weaver gene were investigated by PCR-RFLP and forced PCR-RFLP methods. Allelic and genotypic frequencies are shown by breed in Table 2, as well as homozygosity.
SBWC and XJWC, which implies that allele T may be associated with the milk performance. Hence, dairy utility (XNSN and GZ) than those of wool utility effects on dairy traits. The frequencies of T alternate allele, consisting the XNSN and GZ genetic genotype T (Table 3). Moreover, frequencies of \( P < 0.01 \) (Hardy-Weinberg equilibrium); “\" corresponds to “0.000”. In the TaqI and Hhal analyses, genotypes of goat weaver gene were in Hardy-Weinberg equilibrium except in GZ population. The \( \chi^2 \) parameters of LD were 0.103, 0.139, 0.187 and 0.192 for XNSN, GZ, SBWC and XJWC respectively, which indicated that the two SNPs were not linked strongly in the populations. Genotypic and allelic frequencies in the PCR-RFLP analysis with TaqI, allele T were higher in dairy utility breeds, compared with the down-triangle. The frequencies between the four breeds in the down-triangle. **Table 3. The \( \chi^2 \) (df) from genotypic and allelic frequencies among the four breeds at goat weaver-TaqI and Hhal loci.**

<table>
<thead>
<tr>
<th>Loci</th>
<th>Breed</th>
<th>XNSN</th>
<th>GZ</th>
<th>SBWC</th>
<th>XJWC</th>
</tr>
</thead>
<tbody>
<tr>
<td>TaqI locus</td>
<td>XNSN</td>
<td>6.943 (2)</td>
<td>155.150*** (2)</td>
<td>112.266*** (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GZ</td>
<td>4.377* (1)</td>
<td>239.350*** (2)</td>
<td>175.332*** (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SBWC</td>
<td>160.491*** (1)</td>
<td>214.743*** (1)</td>
<td>0.067 (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>XJWC</td>
<td>109.280*** (1)</td>
<td>144.927*** (1)</td>
<td>0.065 (1)</td>
<td></td>
</tr>
<tr>
<td>Hhal locus</td>
<td>XNSN</td>
<td>3.810 (2)</td>
<td>55.512** (2)</td>
<td>33.821*** (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GZ</td>
<td>0.367 (1)</td>
<td>72.185** (2)</td>
<td>43.998** (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SBWC</td>
<td>53.620*** (1)</td>
<td>62.816*** (1)</td>
<td>0.215 (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>XJWC</td>
<td>32.013*** (1)</td>
<td>37.356*** (1)</td>
<td>0.209 (1)</td>
<td></td>
</tr>
</tbody>
</table>

\( \chi^2 \) (df) represent differences of genotypic frequencies between four breeds in the up-triangle; \( \chi^2 \) (df) represent differences of allelic frequencies between the four breeds in the down-triangle. *\( P < 0.05 \); ***\( P < 0.001 \).

In the TaqI and Hhal analyses, genotypes of goat weaver gene were in Hardy-Weinberg equilibrium except in GZ population. The \( \chi^2 \) parameters of LD were 0.103, 0.139, 0.187 and 0.192 for XNSN, GZ, SBWC and XJWC respectively, which indicated that the two SNPs were not linked strongly in the populations. Genotypic and allelic frequencies in the PCR-RFLP analysis with TaqI and Hhal were found to be different among XNSN dairy, GZ dairy, SBWC wool and XJWC wool based on chi-square tests (\( \chi^2 = 354.862, df = 6, ***P < 0.001 \) and \( \chi^2 = 328.462, df = 3, ***P < 0.001 \) for TaqI; \( \chi^2 = 110.861, df = 6, ***P < 0.001 \) and \( \chi^2 = 95.249, df = 3, ***P < 0.001 \) for Hhal, respectively) (Table 3). Moreover, frequencies of genotypetype T<sub>2</sub>T<sub>2</sub> and H<sub>2</sub>H<sub>2</sub>, allele T<sub>2</sub> and H<sub>2</sub> were higher in dairy utility (XNSN and GZ) than those of wool utility (SBWC and XJWC), which implies that allele T<sub>2</sub> and H<sub>2</sub> may be associated with the milk performance. Hence, caprine weaver gene was considered having positive effects on dairy traits. The frequencies of T<sub>2</sub> and H<sub>2</sub> allele were low in the dairy utility breeds, compared with the alternate allele, consisting the XNSN and GZ genetic background of the breeds. Both breeds were identified as dairy goat breeds about 20 years ago which were needed for further selection during breeding. This assumes that the markers could be useful in breeding strategy in goat by marker-assisted selection. Taken together, the observations suggest that the distributions of genotypic and allelic frequencies of goat weaver gene were significantly associated with different goat utility and different selection diverges affected the frequency of weaver gene that played multiple roles in dairy and wool producing traits.

We previously reported that one SNP located on exon 4 was associated with milk yield (\( P = 0.045 \)) (Li et al., 2010). However, no difference was found in milk composition (\( P > 0.05 \)). In this study, we revealed the association of the TaqI polymorphisms within the 3′UTR of weaver gene with milk yield, protein and solids-not-fat percentage in the XNSN (Table 4). Statistical results were found in milk yield between different genotypes in TaqI polymorphism locus (\( P = 0.015 < 0.05 \), Table 4). Protein and solids-not-fat content were higher in milk
The linkage disequilibrium between the three SNP genotypes was considered strong (Li et al., 2009). The linkage disequilibrium between the two SNPs was estimated, which indicated that the two SNPs were not linked strongly in the analyzed populations ($r^2 = 0.103$). Hence, the TaqI-T2 allele may be beneficial for improving milk yield but not the HhaI-H2 allele. Therefore, NC_007299:g.99045C > T mutation may directly or indirectly influence the stability of the mRNA of weaver, and consequently, the amount of protein produced, which needs further study.

### Table 4. Associations of different genotypes within the weaver gene with milk yield and milk composition in Xinong Sannen dairy goat.

<table>
<thead>
<tr>
<th>Trait</th>
<th>SNP genotype (mean ± S.E.)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T2T2</td>
<td>T1T2</td>
</tr>
<tr>
<td>MY (kg)</td>
<td>628.17 ± 13.66(^a)</td>
<td>624.39 ± 8.14(^a)</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>2.87 ± 0.20</td>
<td>2.68 ± 0.15</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>3.52 ± 0.10(^a)</td>
<td>3.21 ± 0.07(^b)</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>4.13 ± 0.06</td>
<td>4.10 ± 0.04</td>
</tr>
<tr>
<td>SNF (%)</td>
<td>8.76 ± 0.09(^a)</td>
<td>8.60 ± 0.07(^a)</td>
</tr>
<tr>
<td>TS (%)</td>
<td>11.63 ± 0.30</td>
<td>11.40 ± 0.16</td>
</tr>
</tbody>
</table>

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).

Conclusions

To summarize, we firstly reported the TaqI and HhaI polymorphisms in goat weaver gene and their associations with the production traits and milk composition in XNSN dairy goat breed. Some of those with better performance of T2T2 genotype could be used for the breeding of new breeds of dairy goat in China. Moreover, this study contributed to its evaluation as a genetic marker in goat breeding and extension of the spectrum of genetic variation of caprine weaver gene.

ACKNOWLEDGEMENTS

This work was supported by Research Fund for the Doctor Program of Higher Education of China (No.20080712001), the Young Topnotch Researcher Support Project of Northwest A&F University (No.QNGG-2009-007), Natural Science Foundation of Shaanxi Province of China (No. 2011JQ0309), and Special Fund for animal breeding of Northwest A&F University (X.Y. Lan). We thank Professor Jun Luo for collecting the dairy goat blood samples.

REFERENCES


