Genotyping Analysis of Milk Protein Genes in Different Goat Breeds Reared in Egypt

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ABSTRACT

The genes that encode the major protein and whey protein of milk are candidate genes in molecular marker assisted selection to improve the milk productivity in farm animals. Our study concerned with genotyping analysis of four milk genes, CSN1S1, CSN1S2, KCN and β-LG in four goat breed reared in Egypt; Baladi, Barki, Damascus and Zaraibi. Our results revealed that the CSN1S1 allelic variants in tested breeds showed five different genotypes, three of them were homozygous (A/A (4.5%), B/B (6.8%) and D/D (2.3%)) and the other two were heterozygous (A/C (40.9%) and B/D (45.5%)). For CSN1S2 gene, the Egyptian goat breeds carry the A, B and F alleles, while the C, D, E and 0 alleles were not present. The frequency of homozygous genotypes AA, BB were 28.9% and 26.7%, respectively, while heterozygous genotypes AB and AF were 40% and 4.4%, respectively. The k-CN results illustrated that among different Egyptian breeds, B allele was the most common allele in breeds with maximum frequency in Zaraibi breed (90%), where the allele C with low frequency in Barki (9.1%) and Zaraibi (8.3%) breed. Allele A was displayed in different frequencies ranged from 45.5% (Barki) to 10% (Zaraibi). Genetic polymorphism of β-LG exon 7 showed two genetic variants S1S2 genotype with 45.5% and S2S2 with 54.5%. The homozygous S1S1 genotype was not displayed in all tested animals. Among different breeds, the polymorphism within the proximal promoter region and exon 1 of β-LG displayed three genetic variants CC, TT and CT at different frequencies with the exception of CT which was not displayed in Zaraibi breed. In conclusion, genetic analysis of the goat milk protein genes is a valuable tool for selecting the favorable genotype of milk genes with the highest yield of milk.

KeyWords: Goat, polymorphism, milk genes, PCR-RFLP.

INTRODUCTION

The molecular analysis of goat genome is currently carried out using specific goat sequences as well as heterologous ones isolated from cattle and sheep (Vaiman et al., 1996). Deoxyribonucleic acid (DNA) based markers have a number of favorable characteristics which represent ideal tool for studying the genetic quantitative traits (QTLs) (Ajmone-Marsan et al., 2001). The genetic markers applied to animal breeding and production is focused mainly on analysis of genes with economically important QTLs. The genes that encode the major protein and whey protein of milk are candidate genes of milk trait. The association of genetic polymorphism with milk production and composition has stimulated interest in using genetic polymorphism of milk protein genes in molecular marker assisted selection (MAS) to improve milk productivity in farm animals (Ng-Kwai-Hang, 1998).

The analysis of Ca-sensitive caseins (CN) variation in the domesticated goat is quite complex because a large number of mutations involved the 4 coding genes (Rando et al., 2000 and Caroli et al., 2006), which are tightly linked in the CN cluster (Threadgill and Womack, 1990 and Rijnkes, 2002). The three Calcium-sensitive caseins αS1-CN, αS2-CN and β-CN are coded by the CSN1S1, CSN1S2 and CSN2 genes, respectively, whereas Kappa-CN is coded by the CSN3 gene. Deep relationships between the large genetic variation and functional and biological properties affecting milk quality, composition and technological characteristics have been found mainly in goat CSN1S1 (Clark and Sherbon, 2000 and Serradilla, 2002) which is characterized by high quantitative and qualitative variation. In addition, the CSN1S2 and CSN2 genes of the cluster have been associated with differences in the expression level of specific protein (Caroli et al., 2006).

Kappa casein (K-CN) is the milk protein essential for the micelle formation and stabilization and influences the manufacturing properties of milk. It differs from other caseins in its solubility over a broad range of calcium ion concentrations and contains a hydrophilic C-terminal region (Mercier et al., 1973).

Beta-lactoglobulin (β-LG) is the major whey protein in the milk of ruminants and several non-ruminant species (Perez and Calvo, 1995). Schaar, et al. (1985) reported that milk protein genotype has a significant influence on cheese making with β-LG genotype; the effect is expressed mainly in the behavior of milk during renneting.

In Egypt, there are four main goat breeds; Baladi, Barki, Damascus and Zaraibi. Baladi breed is the most dominant indigenous Egyptian goat breeds. It is found in the Delta and...
the Nile Valley and its animals are black; they may be white, red or gray. The Barki breed- it is also named Sahrawi- is found in the Northwestern Coastal desert of Egypt and its animals are mainly black with or without white spots on the head and body. Zaraibi breed- it is also named Nubian or Egyptian Nubian- is found in the North East of the Nile Delta and its animals are cream, red, black or brown (Galal et al., 2005). Damascus breed is not indigenous Egyptian breed; it is raised in the regions of Syria and Lebanon. It is used for crossbreeding with indigenous goat breeds for their genetic improvement primarily for milk production. Its animals are cream, red, black or brown in color and they can be either horned or polled.

This study was aimed to analyze the genotyping of the four milk protein genes CSN1S1, CSN1S2, KCN and β-LG in four goat breeds reared in Egypt; Baladi, Barki, Damascus and Zaraibi; Using PCR-RFLP technique.

**MATERIALS AND METHODS**

**Animals:**
The goats undertaken in this study were selected from distant experimental stations belonging to Animal Production Research Institute, Agricultural Research Center, Desert Research Center, Ministry of Agriculture and Agriculture Faculties; where random-mating strategies are employed. To minimize the likelihood of any close genetic relationships, the number of the breed samples from each station was restricted deliberately. Forty four animals that belonging to the four goat breeds; Baladi (11 goats), Barki (11 goats), Damascus (12 goats) and Zaraibi (10 goats); were undertaken in this study.

**Genomic DNA extraction:**
Genomic DNA was extracted from whole blood of the goat breed samples from each station was restricted deliberately. Genomic DNA was extracted from whole blood of the goat; were undertaken in this study.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer no.</th>
<th>Allele detected</th>
<th>Sequence 5’----------------------------- 3’</th>
<th>Anneal temp. (°C)</th>
<th>PCR product size (bp)</th>
<th>Restriction enzyme</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSN1S1</td>
<td>Primer 1</td>
<td>A, B, C and D</td>
<td>TTC TAA AAG TCT CAG AAG CAG GGG TGT ATA GCC TGT TAT</td>
<td>60</td>
<td>223</td>
<td>XmnI</td>
<td>Yahyaoui et al. (2000)</td>
</tr>
<tr>
<td>CSN1S1</td>
<td>Primer 2</td>
<td>A and B</td>
<td>GGC ATT CAT CCC AGA AAG CTC TTT ATT TGC TTC TTA</td>
<td>54</td>
<td>1300</td>
<td>Msel</td>
<td>Cosenza et al. (1998)</td>
</tr>
<tr>
<td>CSN1S1</td>
<td>Primer 3</td>
<td>A, B and C</td>
<td>AAT TAA CTG CTT CTA CGT AAG AAG AAT GGA GAA</td>
<td>54</td>
<td>3700</td>
<td>PstI</td>
<td>Yahyaoui et al. (1999)</td>
</tr>
<tr>
<td>CSN1S2</td>
<td>Primer 4</td>
<td>0 and D</td>
<td>GAC ACA TAG AGA AGA TTC CGT TGG GAC ATT TTA CTC</td>
<td>51</td>
<td>301</td>
<td>Ncol</td>
<td>Yahyaoui et al. (2001)</td>
</tr>
<tr>
<td>CSN1S2</td>
<td>Primer 5</td>
<td>E</td>
<td>TTT AGG AAC CGA GGA CCA AGT A CTG AAA CTG TAG AGG AAG AAT GAT</td>
<td>56</td>
<td>232</td>
<td>NlaIII</td>
<td>Lagonigro et al. (2001)</td>
</tr>
<tr>
<td>CSN3</td>
<td>Primer 7</td>
<td>A or B and C</td>
<td>TGT GCT GAG TAG GTA TCC TAG TTA TGG GGG TTT TGC TCT TGT ATG TCT TAG</td>
<td>63</td>
<td>459</td>
<td>Alw41</td>
<td>Yahyaoui et al. (2001)</td>
</tr>
<tr>
<td>CSN3</td>
<td>Primer 8</td>
<td>A and E</td>
<td>GCG TGG TCT TTT AGT TCT CTT TAG TCC CAA TGT TGT ACT TTT TTA ACA TC</td>
<td>63</td>
<td>645</td>
<td>HaeIII</td>
<td>Yahyaoui et al. (2003)</td>
</tr>
<tr>
<td>β-LG</td>
<td>Primer 9</td>
<td>S, and S₂</td>
<td>CGG GAG CCT CCC CCT TCT CGG CCT TGT TCG AGT TGT GGT GT</td>
<td>65</td>
<td>426</td>
<td>SacI</td>
<td>Pena et al. (2000)</td>
</tr>
<tr>
<td>β-LG</td>
<td>Primer 10</td>
<td>C and T</td>
<td>GTC ACT TCC CGG TCC TG GCC CTT TCA TGG TCT GGT TGA CG</td>
<td>63</td>
<td>710</td>
<td>SmaI</td>
<td>Yahyaoui et al. (2000)</td>
</tr>
</tbody>
</table>

**Polymerase chain reaction (PCR):**
A PCR cocktail consists of 1.0 μM upper and lower primers specific for each studied gene (Table 1) and 0.2 mM dNTPs, 10 mM Tris (pH 9), 50 mM KCl, 1.5 mM MgCl₂, 0.01 % gelatin (w/v), 0.1 % Triton X-100 and 1.25 units of Taq polymerase was used. The cocktail was aliquoted into tubes with 100 ng DNA of goat. The reaction ran in a Coy Temp Cycler II (Coy Corporation, MI, USA). The reaction was cycled for 1 min at 94°C, 2 min at an optimized annealing temperature that is determined for each primer (Table 1) and 2 min at 72°C for 30 cycles.

**RFLP and agarose gel electrophoresis:**
Twenty μl of PCR product for each primer were digested with 10 units of specific restriction enzyme (Table 1) in a final reaction volume 25 μl. The reaction mixture was incubated at 37°C in water bath over night. After restriction digestion, the restricted fragments were analyzed by electrophoresis on 2.5% agarose/1X TBE gel stained with ethidium bromide. The 100 bp ladder was used as molecular size marker. The bands were visualized under UV light and photographed with yellow filter on black and white film.
RESULTS

Genetic polymorphism of CSN1S1:
The PCR products of primer 1 (223-bp) digested by restriction enzyme XmnI allowed to identify five genotypes. Three of them were homozygous and the other two were heterozygous. The A/A genotype gave a fragment at 150-bp, B/B genotype at 161-bp, D/D genotype at 223-bp (Figure 1). The presence of A/C illustrated by two fragments at 150- and 212- bp and B/D by two fragments at 161- and 223-bp (Figure 2).

Table 2: Genotype frequencies of CSN1S1 among four tested goat breeds

<table>
<thead>
<tr>
<th>Tested breed</th>
<th>No. of animals</th>
<th>A/A (%)</th>
<th>B/B (%)</th>
<th>D/D (%)</th>
<th>A/C (%)</th>
<th>B/D (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baladi</td>
<td>11</td>
<td>-----</td>
<td>----</td>
<td>----</td>
<td>90.9</td>
<td>9.1</td>
</tr>
<tr>
<td>Barki</td>
<td>11</td>
<td>-----</td>
<td>----</td>
<td>----</td>
<td>27.3</td>
<td>72.7</td>
</tr>
<tr>
<td>Damascus</td>
<td>12</td>
<td>16.7</td>
<td>16.7</td>
<td>8.3</td>
<td>33.3</td>
<td>25.0</td>
</tr>
<tr>
<td>Zaraibi</td>
<td>10</td>
<td>-----</td>
<td>10.0</td>
<td>----</td>
<td>10.0</td>
<td>80.0</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>4.5</td>
<td>6.8</td>
<td>2.3</td>
<td>40.9</td>
<td>45.5</td>
</tr>
</tbody>
</table>

Genetic polymorphism of CSN1S2:
A and B allele detection:
The PCR products of primer 2 (1.3-kb) digested by restriction enzyme MseI showed a specific fragment of about 300-bp for allele A, while MseI digestion detected allele B by giving a specific fragment of about 400-bp. In addition to these specific fragments by which we can differentiate between A and B alleles, there were two common fragments appeared at 270- and 230-bp. The AB genotype gave 4 detectable fragments at 400-, 300-, 270- and 230-bp (Figure 3). The results showed that the appearance of A allele in 33 of 44 animals (75.0%), where B allele was displayed in 30 animals (68.2%).

C allele detection:
The PCR products of primer 3 (3.7 kb) digested by restriction enzyme PstI showed three variant genotypes; allele A at sizes 1700-, 900- and 700-bp, allele B at sizes 1700-, 1300- and 700- bp and the genotype AB at sizes 1700-, 1300-, 900- and 700- bp. The results of the two alleles A and B were confirmed as previously mentioned using MseI-RFLP. All

Figure 1: The electrophoretic pattern obtained after digestion of PCR amplified goat CSN1S1 products with XmnI.
M: 100-bp ladder marker.
Lane 1: A/A homozygous genotype.
Lane 2: B/B homozygous genotype.
Lane 3: B/D heterozygous genotype.
Lane 4: A/C heterozygous genotype.

Figure 2: The electrophoretic pattern obtained after digestion of PCR amplified goat CSN1S2 products with MseI.
M: 100-bp ladder marker.
Lane 1: A/C heterozygous genotype.
Lane 2: B/D heterozygous genotype.
Lane 3: D/D homozygous genotype.

Figure 3: The electrophoretic pattern obtained after digestion of PCR amplified goat CSN1S2 products with MseI.
M: 100-bp ladder marker.
Lanes 1, 2 and 6: B/B homozygous genotype.
Lane 3, 4 and 5: A/B heterozygous genotype.
Lane 7: A/A homozygous genotype.
DNA samples extracted from four Egyptian goat breeds were not showed the fragment at 950-bp which is characterized for the C allele.

0 and D allele detection:
The PCR products of primer 4 (301-bp) digested by restriction enzyme NcoI presented two fragments at 168- and 133-bp in all DNA samples. The fragments of 301-bp (specific for 0 allele) and 62-bp (specific for D allele) were not displayed in all samples. This results indicated to the absence of 0 and D alleles in all tested goat animals.

E allele detection:
The PCR products of primer 5 (232-bp) digested by restriction enzyme NlaIII allowed to identify two fragments at 142- and 90-bp in all DNA samples which revealed that the absence of the E allele, where the fragment of 232-bp (specific for E allele) was not displayed in all samples.

F allele detection:
The PCR products of primer 6 (310-bp) digested by restriction enzyme Alw26I showed that all DNA samples (except two samples) gave two fragments at 179- and 131-bp revealed that the absence of the F allele in the most tested animals, where undigested fragment at 310-bp (specific for F allele) was not displayed in 42 samples. Only two samples showed 3 fragments after digestion at 310-, 179- and 131-bp and revealed that these two animals have genotype F/N (Figure 4), where N is any other allele of this locus. According to the results of MseI-RFLP (for detection of A and B alleles), these two animals have a genotype F/A due to the appearance of digested fragments characteristic for A allele in these two DNA samples.

Among different breeds, we found that the allele B is the most common allele in the four tested breeds and appeared with the maximum frequency in Zaraibi breed (90%), where the allele C was appeared with low frequency in Barki (9.1%) and Zaraibi (8.3%) breeds. Allele A was displayed in different frequencies ranged from 45.5% (Barki) to 10% (Zaraibi) (Table 3).

Table 3: A, B and C allele frequencies of CSN3 among four tested goat breeds

<table>
<thead>
<tr>
<th>Tested breed</th>
<th>No. of animals</th>
<th>Allele frequencies (%)</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baladi</td>
<td>11</td>
<td></td>
<td>27.3</td>
<td>72.7</td>
<td>18.2</td>
</tr>
<tr>
<td>Barki</td>
<td>11</td>
<td></td>
<td>45.5</td>
<td>54.5</td>
<td>9.1</td>
</tr>
<tr>
<td>Damascus</td>
<td>12</td>
<td></td>
<td>33.3</td>
<td>63.6</td>
<td>50.0</td>
</tr>
<tr>
<td>Zaraibi</td>
<td>10</td>
<td></td>
<td>10.0</td>
<td>90.0</td>
<td>8.3</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td></td>
<td>29.5</td>
<td>68.2</td>
<td>22.7</td>
</tr>
</tbody>
</table>

Genetic polymorphism of β-lactoglobulin:
S1S1, S1S2 and S2S2 allele detection:
The PCR products of primer 9 (426-bp) digested by restriction enzyme SacII produced two genetic variants, S1S2 genotype gave three fragments at 426-, 349- and 77- bp and S2S2 gave one undigested fragment at 426-bp (Figure 6). The homozygous S1S1 genotype was not displayed in all tested animals. (Table 4) summarized the allele frequencies in the 44 tested animals.
Genotyping Analysis of Milk Protein Genes in Different Goat Breeds Reared in Egypt

Table 5: Genotype frequencies of CC, TT and CT of β-LG among four goat breeds.

<table>
<thead>
<tr>
<th>Tested breed</th>
<th>No. of animals</th>
<th>Genotype frequencies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CC</td>
</tr>
<tr>
<td>Baladi</td>
<td>11</td>
<td>54.5</td>
</tr>
<tr>
<td>Barki</td>
<td>11</td>
<td>72.7</td>
</tr>
<tr>
<td>Damascus</td>
<td>12</td>
<td>25.0</td>
</tr>
<tr>
<td>Zaraibi</td>
<td>10</td>
<td>20.0</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>43.2</td>
</tr>
</tbody>
</table>

DISCUSSION

In caprine breeds, CSN1S1 has 16 alleles associated with different rates of protein synthesis. On the basis of milk content of αS1-CN, the CSN1S1 variants can be grouped into 4 classes: Strong alleles (A, B1, B2, B3, B4, C, H, L and M), producing almost 3.5 g/l of αS1-CN each; intermediate alleles (E and D, 1.1 g/L); weak alleles (F and G, 0.45 g/l) and null alleles (01, 02 and N) apparently producing no αS1 casein (Rando, et al. 2000 and Ramunno, et al. 2005). Our study revealed that the tested breeds showed low percentage of homozygous strong genotype AA (4.5%) and BB (6.8%) of milk casein used for cheese industry. The mild genotype DD recorded very low percentage (2.3%). This genotype is associated with medium level of milk protein favorable for allergic subject (nutritional purpose) especially infant diet where the goat milk with low casein is reported less allergenic than cow’s milk (Roncada, et al. 2002).

At least 7 alleles have been identified at CSN1S2, associated with 3 synthesis levels. The A, B (Boulanger, et al. 1984), C (Bouniol, et al. 1994), E (Lagongro, et al. 2001) and F (Ramunno, et al. 2001) alleles were associated with a normal αS2-CN synthesis level, whereas D and 0 were associated with lower and null synthesis levels, respectively (Ramunno, et al. 2001). The results presented in this study provided that the Egyptian goat breeds carry the A, B and F alleles while the C, D, E and 0 alleles were not present. The frequencies of homozygous genotypes AA, BB were 28.9% and 26.7%, respectively, while heterozygous genotype AB and AF were 40.0% and 4.4%, respectively. Previous study by (Ramunno, et al. 2001) reported that the homozygous genotypes are associated with good quality of milk protein.

The results of CSN1S1 and CSN1S2 in this study indicated that the quality of milk protein in Egyptian goat breeds required for milk industry is not on the level for economic issues because the developmental countries like Egypt needed to fill nutritious gap in milk and milk industry.

Recently, 16 variants of goat k-CN have been identified, involving 15 polymorphic sites in CSN3 exon 4 (Yahyaoui, et al. 2001; Angiolillo, et al. 2002; Yahyaoui, et al. 2003; Jann, et al. 2004 and Prinzenberg, et al. 2005). Among different Egyptian breeds, we found that the allele B is the most common allele in the four tested breeds and appeared with the maximum frequency in Zaraibi breed (90%), where the allele C was appeared with low frequency in Barki (9.1%) and Zaraibi (8.3%) breeds. Allele A was displayed in different frequencies ranged from 45.5% (Barki) to 10% (Zaraibi) (Table 3). This could be explained due to the caprine
k-CN B allele is the ancestral allele, while k-CN C allele is more divergent and the A variant shows an intermediate similarity. The difference between k-CN A and k-CN B involves only one amino acid substitution at position 119, where the valine in variants A is substituted by isoleucine in variant B. The k-CN A differs from the C variant in the following amino acid substitution: Valine for isoleucine at positions 65 and 119, alanine for valine at position 156 and serine for proline at position 159. The first amino acid substitution (position 65) occurs in the N-terminal region (caseino-macropeptide) (Yahyaoui, et al. 2001).

β-LG belongs to the lipocalin protein family, constituted by small secreted proteins which are characterized by their affinity to bind hydrophobic molecules. Although lipocalins have been classified primarily as transport proteins, they are implicated in several biological processes such as retinol and pheromone transport, synthesis of prostaglandins, immune response and cell homeoostasis (Flower, 1996). In our study, 426-bp fragment from exon 7 was amplified by PCR to detect the presence of S1 or S2 variations. Exon 7 of goat β-LG gene comprises most of the 3’ non-coding regions on the mRNA (Folch, et al. 1994). The difference in the mRNA stability have been reported to the cause of a reduction to one-third in the mRNA level of allele E of the goat αs2-casein gene, where E allele is considered as an intermediate allele associated with medium level of protein synthesis. Gene

Also our result showed that the polymorphism within a 710-bp PCR amplified fragment of goat β-LG gene (comprising 588-bp of proximal promoter region and 122-bp of exon 1) was detected. Several transcription factors are known to bind to recognition sequences of the goat β-LG promoter (Watson, et al. 1991 and Folch, et al. 1994). Among tested different breeds in this study, all genetic variants were displayed with exception of CT which was not displayed in Zarabi breed. The genotyping with high frequencies were CC in barki (72.7%), TT in Zarabi (80.0%) and CT in Damascus breed (66.7%) (Table 5).

In conclusion, the goat genetic polymorphism studies related to milk quality and quantity enable us to identify the favorable genotypes related to highest milk yields as well as the milk protein contents that are of most interest because of the direct relationships between milk quality composition and technological characteristic.

REFERENCES


