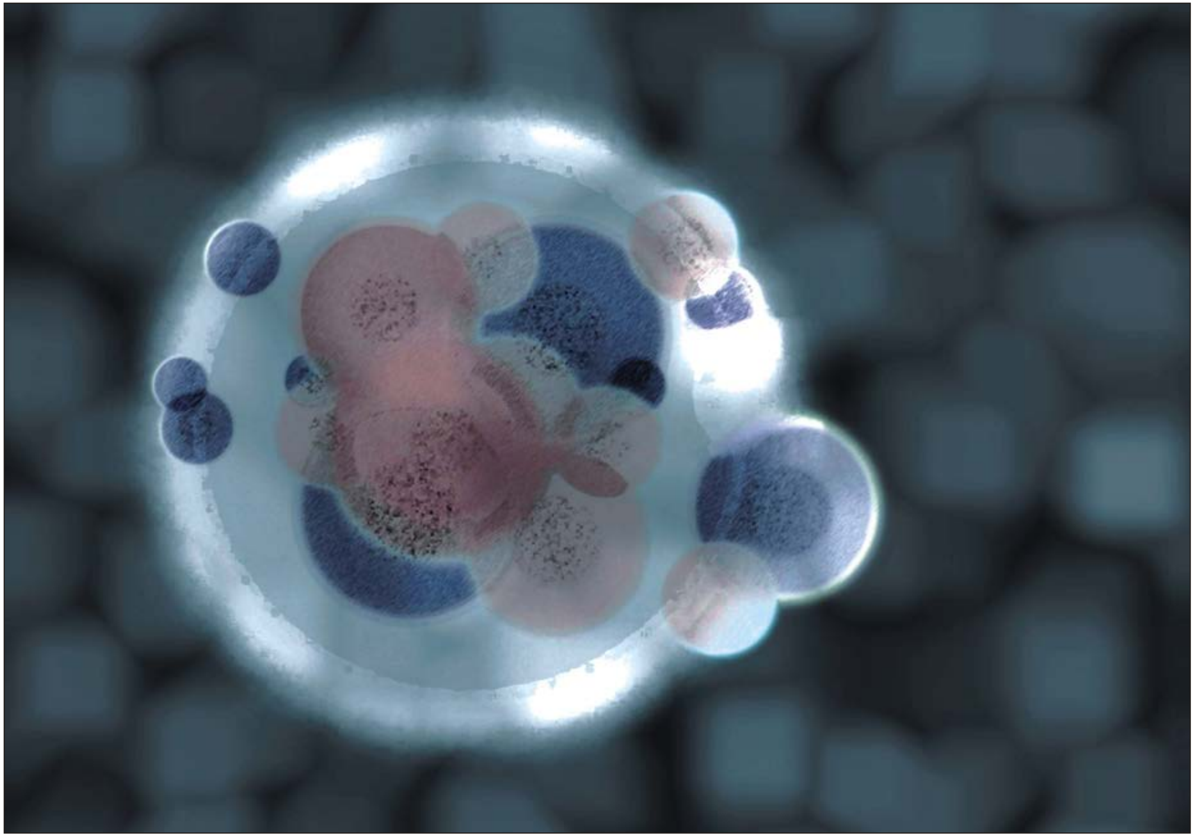

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Analysis of Genetic Polymorphisms in the Egyptian Goat CSN1S2 Using Polymerase Chain Reaction

Othman E. Othman and Sahar Ahmed
Department of Cell Biology, National Research Center, Dokki, Egypt

Abstract: Forty-five animals belonging to four Egyptian goat breeds were undertaken in this study to analyze the different alleles of CSN1S2 gene. The PCR products of five different primers were digested by restriction enzymes *Mse*I, *Pst*I, *Nco*I, *Nla*III and *Alw26*I for detecting the presence of seven alleles A and B, C, D and 0, E and F, respectively. Present results showed that the homozygous genotypes AA and BB were observed at frequencies 28.9 and 26.7%, respectively and the heterozygous genotype AB was displayed in 18 animals (40%) whereas the genotype AF was present in two animals (4.4%). The alleles C, D, E and 0 were not displayed in tested animals. In conclusion, the improving of Egyptian goat breeds towards economic issues should be directed towards increasing the frequency of homozygous genotypes with the high casein content required for milk industry through selecting animals by molecular analysis.

Key words: Goat, CSN1S2, milk protein, PCR, RFLP

INTRODUCTION

The genes that encode the major milk proteins are thought of as candidate genes for the observed variation in protein composition. As an example, cheese yield is related to the casein content in milk, particularly the ratio of casein to the total protein (Pagnacco and Caroli, 1987; Ramunno *et al*., 2001).

In goat milk, four caseins (αs_1, αs_2, β and k-casein) coded by four tightly linked autosomal genes (CSN1S1, CSN1S2, CSN2 and CSN3, respectively) were identified. The four Ca-sensitive caseins of goat exhibit both quantitative and qualitative variations arising from genetic polymorphism in the encoded genes (Ramunno *et al*., 2000, 2001).

The goat CSN1S2 locus is characterized by seven alleles (A, B, C, D, E, F and 0) associated with at least three quantitative levels of the corresponding protein: null ($CSN1S2^{\circ}$), intermediate ($CSN1S2^{D}$) and normal (the remaining five others). Each quantitative level of them has an important usage according to its technological characterizations. For example, the human milk is characterized by the absence of αs_2-casein, therefore milk obtained from CSN1S2 0/0 goats could be more suitable than bovine milk for the production of the humanized milk. Also, the goats have CSN1S2 alleles associated with a high content of casein produce milk characterized by a minor diameter of micelles, significantly higher percentage of protein, fat, total calcium and better parameters for curd firming time, curd firmness and cheese yield compared with goats have alleles associated with null or intermediate levels (Martin *et al*., 1999; Ramunno *et al*., 2000, 2001).

In the present study we analyzed the genetic polymorphisms of Alfa S_2 casein in Egyptian goat for improving the goat breeds towards economic issues.

MATERIALS AND METHODS

Genomic DNA extraction: Genomic DNA was extracted from whole blood of 45 goat animals by phenol-chloroform method described previously (John *et al*., 1991). Ten milliliter of blood taken on EDTA was mixed with 25 milliliter cold sucrose-triton and the volume was completed to 50 milliliter by autoclaved double distilled water. The nuclear pellet was suspended in lysis buffer with sodium dodecyl sulfate and proteinase K and incubated overnight in a shaking water-bath at 37°C.

Nucleic acids were extracted once with phenol, saturated with Tris-EDTA buffer, followed by extraction with phenol-chloroform-isoamyl alcohol (25:24:1) and this was followed by extraction with chloroform-isoamyl alcohol (24:1). To the final aqueous phase, sodium acetate and cold 95 % ethanol were added. The tubes were agitated gently to mix the liquids and a fluffy white ball of DNA was formed. The DNA was finally dissolved in an appropriate volume of 1X TE buffer. DNA concentrations were determined and diluted to the working concentration of 50 ng μL^{-1}, which is suitable for polymerase chain reaction.

Corresponding Author: Dr. Othman El Mahdy Othman, Department of Cell Biology, National Research Center, Dokki, Giza, Egypt Fax: 2023370931

Table 1: Information of the five primers used for the detection of seven different alleles of goat CSN1S2 gene

Alleles	Sequence 5′----------------------- 3′	Annealing temp.	Restriction enzymes
A and B	GCCATTCATCCCAGAAAG		
	CTCTTCATTTGCGTTCCTTA	54°C	*Mse*I
C	AATTAACTGCTTCTACCTGG		
	CTCAGAAAGATTAGGGAAAG	54°C	*Pst*I
D and 0	GACACATAGAGAAGATTC		
	CGTTGGGACATTTTATCT	51°C	*Nco*I
E	TTTAGGAAGCGAGGACCAAGTA		
	CTGAAACTGTAGAAGATAGATT	56°C	*Nla*III
F	TCTCTTGCCATTCAAAACA		
	TGGTCTTTATTCCTCTCT	54°C	*Alw26*I

Polymerase chain reaction (PCR): A PCR cocktail consists of 1.0 μM upper and lower primers and 0.2 mM dNTPs, 10 mM Tris (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin (w/v), 0.1% Triton X-100 and 1.25 units of Taq polymerase. The primers used in this study were designed according to Cosenza *et al.* (1998), Ramunno *et al.* (1999, 2000, 2001), Lagonigro *et al.* (2001) (Table 1). The cocktail was aliquot into tubes with 100 ng DNA of goat. The thermal cycling conditions were: 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 microliter of PCR product were digested with 10 units of each restriction enzyme (Table 1) used in this study 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 AND DISCUSSION

Forty five animals belonging to the four Egyptian goat breeds-Baladi, Damascus, Sahrawi and Zaraibi- were undertaken in this study to analyze the different alleles of CSN1S2 gene for selecting animals with milk favorable characters for cheese industry and nutritional purposes.

***Mse*I PCR-RFLP to detect A and B alleles:** To detect A and B alleles, we used the PCR-RFLP technique in which the amplified fragment (1.3 kb) was digested by *Mse*I restriction enzyme and resolved using gel electrophoresis. Allele A showed a specific fragment of about 300 bp while *Mse*I digestion detected the 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 after digestion at sizes about 270 and 230 bp (Fig. 1). AA genotype gave 3 easily-detected fragments of about 300, 270 and 230 bp, BB genotype gave 3 fragments of about 400, 270 and 230 bp while AB genotype gave 4 easily-detected fragments of about 400, 300, 270 and 230 bp. The results showed that the appearance of A allele in 33 of 45 animals (73.3%), where B allele was displayed in 30 animals (66.7%).

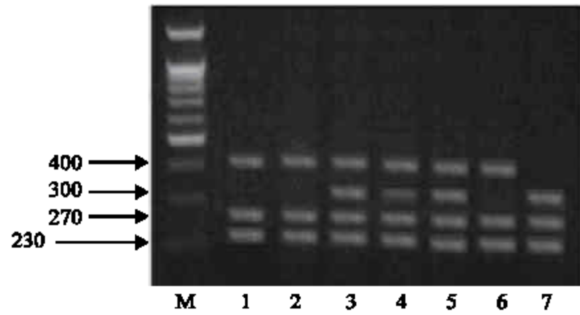

Fig. 1: The electrophoretic pattern obtained after digestion of PCR amplified goat CSN1S2 products with *Mse*I
M: 100 bp ladder marker
Lanes 1, 2 and 6: BB homozygous genotype
Lanes 3, 4 and 5: AB heterozygous genotype
Lane 7: AA homozygous genotype

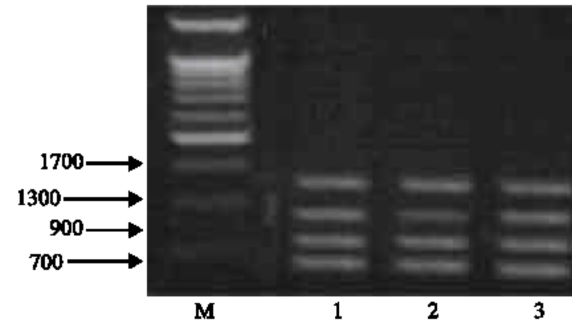

Fig. 2: The electrophoretic pattern obtained after digestion of PCR amplified goat CSN1S2 products with *Pst*I
M: λ DNA-Hind III digest marker
Lanes 1, 2 and 3: AB heterozygous genotype

***Pst*I PCR-RFLP to detect C allele:** The *Pst*I restriction pattern of the amplified fragment was characterized by three variant fragments of about 900 bp (allele A), 1300 bp (allele B) and 950 bp (allele C) and there were two invariants fragments of about 700 and 1700 bp. The results of the two alleles A and B were confirmed as previously mentioned using *Mse*I PCR-RFLP. All DNA

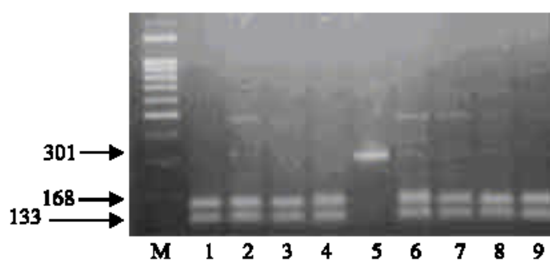

Fig. 3: The electrophoretic pattern obtained after digestion of PCR amplified goat CSN1S2 products with *Nco*I
M: 100 bp ladder marker
Lanes 1-4 and 6-9: N/N genotype, where N = A, B, C, E or F allele
Lanes 5: 301-bp undigested fragment

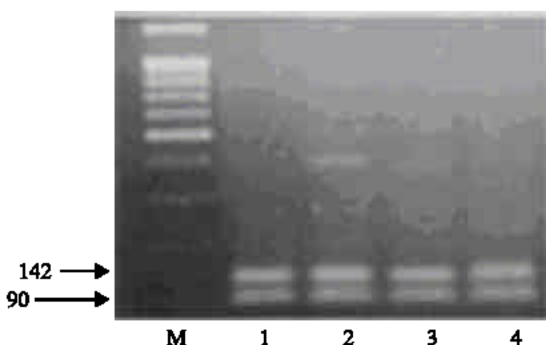

Fig. 4: The electrophoretic pattern obtained after digestion of PCR amplified goat CSN1S2 products with *Nla*III
M: 100 bp ladder marker
Lanes 1-4: not E/not E genotype

samples extracted from four Egyptian goat breeds not showed the fragment of about 950 bp which is characterized for the C allele (Fig. 2) revealed that the absence of the C allele in all tested goat animals.

***Nco*I PCR-RFLP to detect 0 and D alleles:** To detect 0 and D alleles, we used a PCR-RFLP in which the amplified 301 bp fragment was digested by *Nco*I restriction enzyme. The digestion of the amplified PCR products by this enzyme shows an undigested fragment of 301 bp for $CSN1S2^0$ allele, two fragments of 133 and 62 bp for $CSN1S2^D$ allele and two fragment of 168 and 133 bp for the other alleles (A, B, C, E or F).

All DNA samples extracted from four Egyptian goat breeds gave two fragments of 168 and 133 bp (Fig. 3) revealed that the absence of the 0 and D alleles in all tested goat animals, where the fragments of 301 bp (specific for 0 allele) and 62 bp (specific for D allele) were not displayed in all samples.

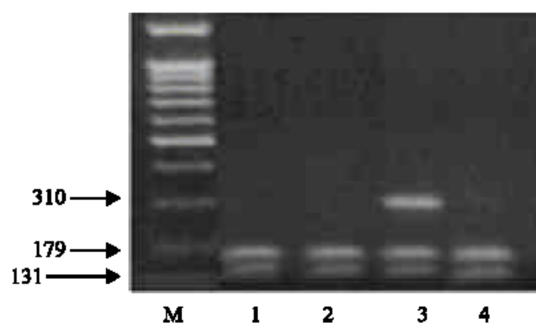

Fig. 5: The electrophoretic pattern obtained after digestion of PCR amplified goat CSN1S2 products with *Alw26*I
M: 100 bp ladder marker
Lanes 1, 2 and 4: N/N genotype, where N = A, B, C, D, E or 0
Lane 3: F/N genotype, where N = A, B, C, D, E or 0

***Nla*III PCR-RFLP to detect E allele:** We amplified the sixteenth exon using the specific primers and digested it with the restriction enzyme *Nla*III. The digestion yielded bands of the expected length, one undigested band at 232 bp for homozygous E/E, two digested bands at 142 and 90 bp for homozygous not E/not E and 3 bands at 232, 142 and 90 bp for heterozygous E/not E. All DNA samples extracted from four Egyptian goat breeds gave two fragments of 142 and 90 bp (Fig. 4) revealed that the absence of the E allele in all tested goat animals, where the fragment of 232 bp (specific for E allele) was not displayed in all samples.

***Alw26*I PCR-RFLP to detect F allele:** The specific primers of F allele amplified a 310-bp fragment of goat αs_2-casein gene from goat DNA genomic samples using PCR. Digestion of the amplified fragment by *Alw26*I restriction enzyme showed an undigested fragment of 310 bp for the $CSN1S2^F$ allele and two fragments of 179 and 131 bp for the other alleles of this locus.

The result showed that all DNA samples (except two samples) gave two fragments of 179 and 131 bp (Fig. 5) revealed that the absence of the F allele in the most tested goat animals, where the undigested fragment of 310 bp (specific for F allele) was not displayed in 43 samples. Only two samples showed 3 fragments after digestion, 301, 197 and 131 bp revealed that these two animals have a genotype F/N, where N is any other allele of this locus. According to the results of *Mse*I PCR-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.

The results presented in this study provided that Egyptian goat breeds carry the A, B and F alleles with 51.1, 46.7 and 2.2%, respectively. The C, D, E and 0 alleles were not present. The frequency of homozygous genotypes AA and BB were 28.9 and 26.7%, respectively while heterozygous genotypes 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 proteins, this indicated that the quality of milk protein for Egyptian goat breeds required for milk industry is not on the level for economic issues in developmental countries like Egypt needed to fill nutritious gap in milk and milk industry.

According to these findings, the improving of Egyptian goat breeds should be directed towards increasing the frequency of homozygous genotypes with high casein content required for milk industry through selecting animals by molecular analysis. Another direction should be focused on casein alpha 2 alleles with low percentage of milk protein, where the goat milk with low casein is favorable in allergic subjects (for example, the infant diet) more than cow's milk (Martin *et al.*, 1999; Ramunno *et al.*, 2001; Roncada *et al.*, 2002). So that, we suggest that producing milk with low protein content required can be applied by importing animals carrying allele characteristic by low milk protein to Egyptian goat breeds. This is more benefit and has influence in economic issues on the long run rather than imported milk or milk products required for infants and other nutritional purposes.

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