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RESEARCH ARTICLE

Genetic Variability of *CSN1S1* Gene in Goat Populations Raised in Southeastern Region of Turkey

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Summary

The objective of this study was to investigate genetic variability of *CSN1S1* gene coding for alpha-s1-casein in goat populations raised in Southeastern Region of Turkey. Blood samples were collected from goats raised in Kilis(n=60), Sanliurfa (n=56), and Siirt (n=55) provinces of Turkey. From the blood samples DNA was isolated by using phenol-chloroform extraction. Genotypes of animals were determined by using polymerase chain reaction (PCR), allele specific PCR or PCR and restriction fragment length polymorphism methods. In Kilis and Sanliurfa populations *CSN1S1* A*, B*, F and N alleles were observed, while in Siirt population only A* and B* alleles were found. Frequencies of A*, B*, F and N alleles were 0.375, 0.367, 0.017 and 0.242 in Kilis, 0.632, 0.208, 0.009 and 0.151in Sanliurfa and 0.782, 0.218, 0.000 and 0.000 in Siirt populations, respectively. *CSN1S1* E and 01 alleles were not observed among the populations studied. Observed and expected genotype frequencies did not differ significantly (P>0.05). The results of this study suggested that there were sufficient genetic variability of *CSN1S1* gene especially in Sanliurfa and Kilis populations in order to select individuals for different breeding purposes.

Keywords: *Goat, Casein, CSN1S1, Polymorphism*

Güneydoğu Anadolu Bölgesi'nde Yetiştirilen Keçilerde Alfa-s1-Kazein (*CSN1S1*) Genindeki Genetik Çeşitlilik

Özet

Bu çalışmanın amacı güneydoğu anadolu bölgesinde yetiştirilen keçilerde alfa-s1-kazeini kodlayan *CSN1S1* genindeki çeşitliliğin araştırılmasıdır. Türkiye'nin Kilis (n=60) Şanlıurfa (n=56) ve Siirt (n=55) illerinde yetiştirilen keçilerden kan örnekleri toplanmıştır. Kan örneklerinden fenol-kloroform yöntemi ile DNA izolasyonu yapılmıştır. Keçilerin genotipleri polimeraz zincir reaksiyonu (PCR), allel spesifik PCR ve kesim bölgesi polimorfizmi yöntemleri ile belirlenmiştir. Kilis ve Şanlıurfa populasyonlarında A*, B*, F ve N allelleri gözlenirken Siirt populasyonunda sadece A* ve B* allelleri bulunmuştur. A*, B*, F and N allellerinin frekansları sırasıyla Kilis keçilerinde 0.375, 0.367, 0.017 ve 0.242, Şanlıurfa populasyonunda 0.632, 0.208, 0.009 ve 0.151, Siirt populasyonunda ise 0.782, 0.218, 0.000 ve 0.000, rolarak hesaplanmıştır. İncelenen populasyonlarda *CSN1S1* E ve 01 allelleri gözlenmemiştir. Beklenen ve gözlenen genotip frekansları arasında önemli bir farklılık bulunmamıştır (P>0.05). Çalışma sonucunda özellikle Kilis ve Şanlıurfa populasyonlarında değişik yetiştirme hedefleri açısından seleksiyon yapılabilecek düzeyde genetik çeşitliliğin bulunduğu kanaatine varılmıştır.

Anahtar sözcükler: *Keçi, Kazein, CSN1S1, Polimorfizm*

INTRODUCTION

Casein genes polymorphism in goats has been extensively investigated due to the less allergenicity of goat's milk than that of cows [1]. Therefore a considerable amount of data on the structure and diversity of casein genes in goat has accumulated in the literature [2,3]. The structure of the *CSN1S1* gene coding for the alfa-S1-Casein (αs1-Cn) has been studied in detail by several researchers [3-5]. *CSN1S1* gene is highly polymorphic and at least 17 alleles of this gene have been detected to date. The alleles are characterized by single nucleotide substitutions, and insertions or deletions [6]. This high poly-

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morphism might be originated partly by inter allelic recombination events [3,7]. The polymorphism of this gene affects casein and protein content of goat milk. Goat *CSN1S1* gene represents an excellent model for demonstrating that the major part of the variability observed in the αs1-Cn content in the goat milk is due to the presence of autosomal alleles at a single structural locus [3]. Based on the content of αs1-Cn in goat milk the *CSN1S1* variants can be classified into four groups: strong alleles (A, B_1, B_2, B_3, B_4, C, H, L and M) each producing nearly 3.5 g/L; intermediate alleles (E and I) each producing 1.1 g/L; weak alleles (D, F and G; 0.45 g/L); and null alleles (0_1, 0_2 and N) producing no αs1-Cn [3-5,7-10]. Content of αs1-Cn in goat milk is highly correlated with individual milk components, thereby with the coagulation properties of the milk. Milk containing higher amount of αs1-Cn has higher total solid and protein and better cheese making properties such as shorter coagulation time and firmer curd [11,12].

Goat and goat milk production still plays an important role for the economy of especially south-eastern region of Turkey. In this region goat milk is produced for cheese making and for producing ice-cream. Breeding of goats producing milk for special consumer needs, i.e. for infant nutrition or nutrition of individuals allergic to cow's milk, would contribute to economical development of the region and to maintain genetic resources of goat in Turkey. Therefore determining the genetic variability of casein genes in goat populations raising in this region might help develop breeding or conservation strategies [13]. There are some reports on the genetic variability of casein genes in goat populations in Turkey. Some research groups have investigated the genetic variability of casein genes (*CSN1S1, CSN1S2, CSN2* and *CSN3*) in Angora and Hair goats raised in Turkey [14,15]. Another research group has investigated the presence and distribution of O and D alleles of *CSN1S2* gene (αs2-casein) in goat populations raised in southeastern region of Turkey [16]. However there is no report on genetic variability of *CSN1S1* gene in goat populations raised in southeastern region of Turkey.

The objective of this study was to investigate genetic variability of *CSN1S1* locus in goat populations raised in Sanliurfa, Kilis and Siirt provinces of Turkey.

MATERIAL and METHODS

Animal Material

Blood samples were collected from goats raised in Kilis (n=60), Sanliurfa (n=56), and Siirt (n=55) provinces of Turkey. Kilis goat has been developed by crossing native Hair goats with Aleppo goats and by subsequent inter-breeding among the crossbred generations. This goat has been considered a separate breed. Kilis goats are distributed especially in the Kilis province and also raised in the provinces along the Syrian border of Turkey. They are kept in small flocks of 2 to 10 goats for particularly milk production. The samples collected from the Sanliurfa province included Aleppo goats, Hair goats and the crossbred animals of these two breeds. The samples from the Siirt province included native Hair goats and their crossbred animals with Angora goats.

Genotyping of the Animals

Genomic DNA was isolated using proteinase-K digestion and phenol-chloroform extraction method [17]. Concentration of the DNA samples were measured by using spectrophotometer and diluted to an end concentration of 100 ng/μL. Sequences of the primers used for amplification of the target region of *CSN1S1* locus were shown in *Table 1*.

The *CSN1S1* F allele is characterized by a deletion of cytosine at the 23th nucleotide of the 9th exon and two insertions of 11 and 3 bp length in the subsequent intron [3,4]. In order to detect A* (A, G, I, H and 02), B* (B_1, B_2, B_3, B_4 C and D), F and N alleles a polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method was used [9]. PCR was carried out in 25 μL volume containing: 100 ng of genomic DNA, 10 pmol of each primer, dNTPs each 0.2 mM, 1.25 U of Taq DNA polymerase (Fermentas, Vilnius, Lithuania), 3 mM $MgCl_2$ and 2.5 μl of 10X reaction buffer contained 100 mM Tris- HCl (pH 8.8) 500 mM KCl and 0.8% Nonidet P40. The amplification protocol consisted of an initial denaturing step of 94°C for 5 min, followed by 10 cycles of 94°C for 30 s, 60°C for 30 s decreasing 1°C in each cycle and 72°C for 30 s and 25 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 30 s. In the final cycle, the extension step was carried out at 72°C for 10 min.

A 10 μL PCR product was digested with the enzyme *Xmn*I according to instructions of the manufacturers (Fermentas, Vilnius, Lithuania). Restriction products were examined on 4% agarose gels stained with ethidium bromide.

The *CSN1S1* E allele carries a truncated long interspersed repeated element (LINE) of 475 bp length within the 19th exon [5]. A direct PCR was used for detecting 475 bp LINE element characterizing *CSN1S1* E allele by using the primer pair given in *Table 1* [18]. For *CSN1S1* E allele a 662 bp PCR product was expected while for other alleles a 205 bp PCR product was amplified. The protocol consisted of an initial

Table 1. *Primer sequences used for detecting different alleles*
Tablo 1. *Farklı allelleri tespit etmek için kullanılan primerlerin baz dizileri*

Allele	Primer Name	Sequence (5'-3')	Reference
A, B, F, N	CSN1S1-F	TTCTAAAAGTCTCAGAGGCAG	9
	CSN1S1-R	GGGTTGATAGCCTTGTATGT	
E and non E	CSN1S1E-F	ATGGGATTGAAAATTCCATGC	18
	CSN1S1E-R	ATACTACTGGAATTTAGGTA	
01 and non 01	AS1a	CCCCAGCTGGTAATGTTTTA	19
	AS1b	GGTCCATCAATTCCCTGTGT	
	AS1c	TGTATGGATCCCTGATTCCTTC	

step of 94°C for 3 min; followed for 30 cycles of 94°C for 30 sec, 59°C 30 sec and 72°C for 30 sec and a final step of 72°C for 3 min. The PCR products were examined on 2% agarose gels stained with ethidium bromide.

The *CSN1S1* 01 allele is characterized by a 8.5 kb deletion spanning from the 12th to 19th exons [10]. For detection of the *CSN1S1* 01 allele an allele specific PCR reaction protocol was used [19]. By using these primers a PCR product of 249 bp fragment length was expected for *CSN1S1* 01 allele while a 281 bp PCR product for alleles other than *CSN1S1* 01 was amplified. The amplification protocol consisted of an initial denaturation step at 94°C for 3 min followed by 40 cycles of 94°C for 30 sec, 62°C for 30 sec and 72°C for 1 min. The final extension step was carried out at 72°C for 3 min. The PCR products were examined on 2% agarose gels stained with ethidium bromide.

Data Analysis

Direct counting was used to estimate allele, haplotype and genotype frequencies. Chi-square statistic (χ^2) was used to check whether the populations were Hardy-Weinberg equilibrium. All statistical analyses were performed using GENALEX 6. software package [20].

RESULTS

Restriction products of some samples after *Xmn*I digestion on agarose gel were shown in *Fig. 1*. Allele and haplotype frequencies after *Xmn*I digestion of PCR products were shown in *Table 2*. All four haplotypes were present in Kilis and Sanliurfa populations, while only D^-I^+ and D^-I^- haplotypes were observed in Siirt population. PCR amplification for detecting *CSN1S1* E allele yielded only a single band of 205 bp fragment size *(Fig. 2)* while PCR amplification for detecting *CSN1S1* 01 allele resulted in a single 281 bp fragment *(Fig. 3)*. Therefore CSN1S1 E and 01 alleles were not found in the three populations studied. In Kilis and Sanliurfa populations *CSN1S1* A* (A, G, I, H and 02) , B* (B_1, B_2, B_3 B_4 C and D), F and N alleles were observed, while in Siirt population only A* and B* alleles were found. Allele frequencies in the three populations were shown in *Table 3*. Observed genotype frequencies were given in *Table 4*. Observed and expected genotype frequencies did not differ significantly (P>0.05).

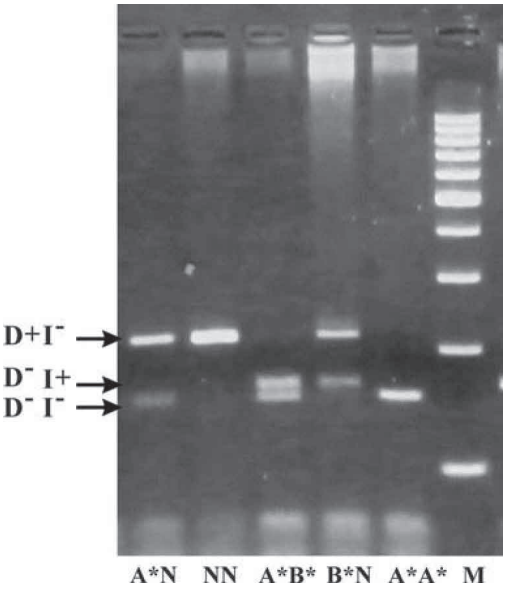

Fig 1. *Xmn*I restriction products of some samples from Sanliurfa population. The haplotypes assigned were shown at the left side of the figure. The genotypes of the individuals assigned were shown at the bottom of the figure. M: Molecular size marker (100 bp ladder)

Şekil 1. Şanlıurfa populasyonundan bazı örneklere ait *Xmn*I enzimi ile kesim ürünleri. Tepit edilen haplotipler resmin sol tarafında gösterilmiştir. Her bir birey için tespit edilen genotipler resmin alt kısmında gösterilmiştir. M: Moleküler markör (100 bp ladder)

DISCUSSION

There are some reports on the genetic variability of casein genes in goat populations in Turkey. The genetic variability of casein genes (*CSN1S1*, *CSN1S2*, *CSN2* and *CSN3*) in Angora and Hair goats raised in Turkey were studied by

Table 2. *Allele and haplotype frequencies after XmnI digestion of PCR products*
Tablo 2. *XmnI ile kesimin ardından tespit edilen allel ve haplotiplerin frekansları*

Locus	Kilis (n=60)	Sanliurfa (n=56)	Siirt (n=55)	All Populations (N=171)
D^+	0.258	0.161	0.000	0.143
D^-	0.742	0.839	1.000	0.857
I^+	0.383	0.214	0.218	0.275
I^-	0.617	0.786	0.782	0.725
Haplotype				
D^+I^+	0.017	0.009	0.000	0.009
D^+I^-	0.241	0.152	0.000	0.135
D^-I^+	0.367	0.205	0.218	0.266
D^-I^-	0.375	0.634	0.782	0.590

D^+ or D^-: *Presence or absence of the cytosine deletion at 23th nucleotide of the 9th exon,* ***I^+ or I^-:*** *Presence or absence of 11 bp insertion at in the 9th intron*

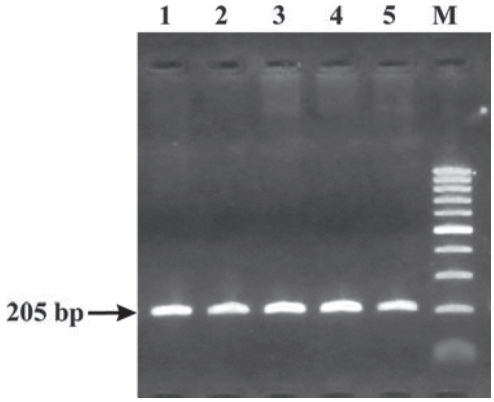

Fig 2. PCR products of some samples from Kilis population for detecting *CSN1S1* E allele (1-5). M: Molecular size marker (100 bp ladder)

Şekil 2. Kilis populasyonundan bazı örneklere ait *CSN1S1* E allelini tespit etmek için uygulanan PCR işlemine ait ürünler M: Moleküler markör (100 bp ladder)

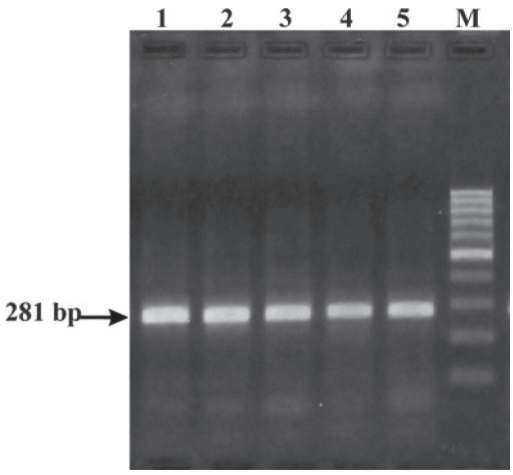

Fig 3. PCR products of some samples (1-5) from Kilis population for detecting 01 allele. M: Molecular size marker (100 bp ladder)

Şekil 3. Kilis populasyonundan bazı örneklerin *CSN1S1* 01 allelini tespit etmek için uygulanan PCR işlemine ait ürünleri M: Moleküler markör (100 bp ladder)

Table 3. *Allele frequencies of CSN1S1 gene in Kilis, Sanliurfa and Siirt goat populations*
Tablo 3. *Kilis, Şanlıurfa ve Siirt'te yetiştirilen keçilerde CSN1S1 geninin allel frekansları*

Allele	Kilis (n=60)	Sanliurfa (n=56)	Siirt (n=55)	All Populations (N=171)
A*	0.375	0.634	0.782	0.590
B*	0.367	0.205	0.218	0.266
E	0.000	0.000	0.000	0.000
F	0.017	0.009	0.000	0.009
N	0.241	0.152	0.000	0.135
01	0.000	0.000	0.000	0.000

Table 4. *Genotype frequencies of CSN1S1 gene in Kilis, Sanliurfa and Siirt populations*
Tablo 4. *CSN1S1 geninin Kilis Şanlıurfa ve Siirt populasyonlarındaki genotip frekansları*

Genotip	Kilis (n=60)	Sanliurfa (n=56)	Siirt (n=55)	All Populations (N=171)
A*A*	0.182	0.429	0.582	0.391
A*B*	0.232	0.286	0.400	0.304
A*F	0.000	0.000	0.000	0.000
A*N	0.150	0.125	0.000	0.094
B*B*	0.170	0,036	0.018	0.077
B*F	0.017	0.000	0.000	0.006
B*N	0.150	0.053	0.000	0.070
FF	0.000	0.000	0.000	0.000
FN	0.017	0.018	0.000	0.012
NN	0.082	0.053	0.000	0.046

several research groups [14,15]. The presence and distribution of O and D alleles of *CSN1S2* gene in goat populations raised in southeastern region of Turkey was also investigated [16]. Another research group studied *Sac*II restriction polymorphism of *CSN3* gene in Honamli, Hair and Saanen goats raised in Burdur vicinity [21]. This is the first report on the genetic variability of *CSN1S1* gene in goat populations raised in southeastern region of Turkey.

The PCR-RFLP method used in this study has also been used by other research groups [2,14]. Haplotype $D^{-}I^{-}$ is associated with *CSN1S1* A* and 01 alleles, haplotype $D^{-}I^{+}$ is associated with B* and E alleles, haplotype $D^{+}I^{+}$ is associated with F allele and haplotype $D^{+}I^{-}$ is associated with N allele [2,3]. Since no *CSN1S1* 01 allele was detected haplotype $D^{-}I^{-}$ was accepted as *CSN1S1* A* allele. On the other hand haplotype $D^{-}I^{+}$ was considered B* allele, as no E allele was detected.

Different allele frequencies were observed among the populations studied. *CSN1S1* F and N alleles were found with different frequencies in Kilis and Sanliurfa populations, while these alleles were not detected in Siirt population. The results found for Siirt population were similar to those reported for Angora and Hair goats raised in Turkey [14]. In contrast to the present study the *CSN1S1* E and F alleles have been found in higher frequencies in other goat breeds raised in different European countries [2,22].

In the present study *CSN1S1* E and 01 alleles were not observed among the populations examined. These alleles have been observed with different frequencies in other goat populations [2,18,22-28].

The *CSN1S1* F allele is characterized by a deletion of cytosine at the 23th nucleotide of the 9th exon and two insertions of 11 and 3 bp length in the subsequent intron [3,4]. The single nucleotide deletion in exon 9 and the two insertions in the ninth intron might be responsible for the alternative skipping of the exons 9, 10, 11 which reduces mRNA level transcribed from F allele [4]. In a study the lowest αs1-casein content has been found in Saanen breed in which *CSN1S1* E and F alleles segregate with a high frequency [29].

The *CSN1S1* N allele is characterized by a deletion of cytosine at the 23th nucleotide of the 9th exon without the insertion of 11 and 3 bp in the subsequent intron, which is present on *CSN1S1* F allele. The one nucleotide deletion results in a frame shift leading to a stop codon at 12th exon. On the other hand this deletion might also affect the splicing mechanism and thereby reduce the mRNA level transcribed from *CSN1S1* N allele [3,4]. The amount of mRNA transcribed from *CSN1S1* N allele is 1/3 of that transcribed from *CSN1S1* F allele and might explain the apparent absence of αs1-Cn in the milk samples of goats homozygote for the *CSN1S1* N allele [3].

These results indicated that both "strong" and "weak" or "null" alleles of *CSN1S1* in terms of *α*s1-Cn content in goat milk were segregating in Kilis and Sanliurfa goat populations. The results of this study suggested that there were sufficient genetic variability of *CSN1S1* gene especially in Sanliurfa and Kilis populations in order to select individuals for different breeding purposes. Goat's milk containing low level of αs1-Cn might reduce intestinal and systemic sensitization to β-lactoglobulin [30]. Human milk contains low level of αs1-Cn [31]. Therefore goats' milk containing low level of αs1-Cn (homozygotes for *CSN1S1* N allele) might be used for preparing "humanized" milk for infant nutrition. On the other hand goats' milk containing higher level of αs1-Cn (ie. *CSN1S1* A*A* or B*B*) might be preferred for cheese production [12]. Further studies are required in order to assess the effect of *CSN1S1* variants on the milk casein content of goat populations raised in Kilis and Sanliurfa goat populations. However CSN1S1 locus is closely linked to, casein genes *CSN1S2*, *CSN2* and *CSN3* and alleles of these loci are inherited together as allele groups called haplotype [32,33]. Therefore future studies should consider not *CSN1S1* gene alone but also other casein genes.

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