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Genetic polymorphism of *CSN1S2* in South African dairy goat populations

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Abstract

Alpha-s_2 casein has a significant influence on protein content in goat milk, and the technological properties important for cheese processing. Specific alleles (A, B, C, E and F) of the alpha $(\alpha)_{s2}$-casein gene (*CSN1S2)* result in higher protein, casein and fat content, and improved coagulation properties, which are useful for improved cheese making. The aim of this study was to investigate the polymorphism and genetic variation of *CSN1S2* in South African dairy goats, using DNA sequencing technology. Sixty dairy goats (20 Saanes, 20 British Alpine, and 20 Toggenburg) and 20 meat-type goats were sequenced with four primers to distinguish among the seven known alleles for α_{s2}-casein. A total of four alleles (A, B, C and F) for *CSN1S2* were observed among the dairy- and meat-type populations with ten genotypes across the populations. The A allele and the AA genotype were the most frequent across the populations, with the favourable AC genotype being the most frequent (0.300) in the Saanen population. Two unique genotypes were detected in the Toggenburg (BB and BF) and one in the meat-type goats (CF). The results indicate moderate genetic variation for α_{s2}-casein in the South African goat populations (42.3–63.6%). Low positive F_{ST} values suggest limited inbreeding. This study confirmed the presence of favourable alleles in the South African goat populations, indicating room for genetic improvement using directional selection for favourable genotypes.

Keywords: alpha-s_2-casein, genetic variation, goat milk, protein content, Saanen
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Introduction

Caseins constitute the largest percentage (80%) of milk proteins in ruminant milk (Haug *et al*., 2007). The casein genes are arranged in a 250 kb cluster on chromosome 6 in both cattle and goats (Ferretti *et al*., 1990; Threadgill & Womack, 1990), with the four genes organized in the order of alpha $(\alpha)_{s1}$-casein, beta (β)-casein, alpha $(\alpha)_{s2}$-casein, and kappa (κ)-casein (Caroli *et al*., 2006; Selvaggi *et al*., 2014). Among caseins, α_{s2}-casein accounts for approximately 20% of the total casein fraction in milk, compared with the 6% contribution of α_{s1}-casein (Selvaggi *et al*., 2014).

The differences in α_{s2}-casein content in milk are associated with the unique physicochemical characteristics of goat caseins and influence the technological behaviour of goat milk during processing into cheese (Selvaggi & Tufarelli, 2011). Goat milk that is characterized by favourable alleles results in a higher content of protein, casein and fat, and improved coagulation properties (Zullo *et al*., 2005). The α_{s2}-casein gene (*CSN1S2*) has seven alleles associated with three levels of synthesis. The favourable A, B, C, E, and F alleles are associated with a high level of α_{s2}-casein in milk, and produce 2.5g α_{s2}-casein/litre per allele (Marletta *et al*., 2007), whereas the rare defective D allele results in a reduction in α_{s2}-casein content, and allele O results in an absence of α_{s2}-casein in milk (Marletta *et al*., 2007; Chessa *et al*., 2008). Favourable haplotypes for the various caseins, including *CSN1S2*, have been identified in a number of breeds, with a positive association with milk quality and technological properties (Dagnachew & Ådnøy, 2014; Vacca *et al*., 2014).

A number of studies have been conducted on caseins in dairy goat breeds found in Mediterranean countries (Sacchi *et al*., 2005, Gigli *et al*., 2008; Vacca *et al*., 2014) owing to the economic importance of goat milk in these countries. The demand for goat milk and goat milk products has increased rapidly in the last decade because of the belief that goat milk possesses unique biologically active, therapeutic and health-promoting properties (Liang & Devendra, 2014). Goat milk is also more digestible than cow milk, which makes it more suitable for infants, small children, and adults that suffer from milk allergies and gastro-

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intestinal problems (Silanikove *et al*., 2010; Amigo & Fontecha, 2011; El-Agamy, 2011; Selvaggi *et al*., 2014).

The genetic characterization of α_{s2}-casein in the South African dairy goat industry is of interest to gain insight into the genetic variability of the gene and identify potential favourable genotypes. The dairy goat industry in this country is small compared with other goat milk-producing countries in the world. However, it is a growing industry, with an emerging market for goat milk and goat milk products, primarily a variety of cheeses. South Africa has approximately 4000 registered dairy goats (Bosman, 2014), which include three commercial breeds, namely Saanen, British Alpine, and Toggenburg. In rural areas, local goat types that are not selected for milk traits are often milked for household purposes.

Research on casein genes in South Africa has been limited to a study on k-casein in indigenous goats (Scheepers *et al*., 2010). The aim of this study was therefore to investigate the polymorphism and genetic variation of the α_{s2}-casein gene (*CSN1S2*) in South African dairy goat populations, including three dairy breeds and a local meat type, using DNA sequencing technology.

Materials and Methods

A total of 80 goats were sampled. Ethical approval was granted by the Animal Ethics Committee (AEC) at the University of Pretoria (UP) (project number: EC088-12 and EC104-13). Blood samples of 80 unrelated goats were collected from five purebred herds across South Africa, including 20 Saanens, 20 British Alpines, 20 Toggenburgs, and 20 meat-type goats. The blood samples were collected from the University of Pretoria's experimental farm (Saanens) and commercial goat farms in the provinces of Gauteng (Toggenburg), North West (British Alpine and Toggenburg), Limpopo (meat-type goats), and Western Cape (Saanen, British Alpine and Toggenburg) in South Africa (Figure 1).

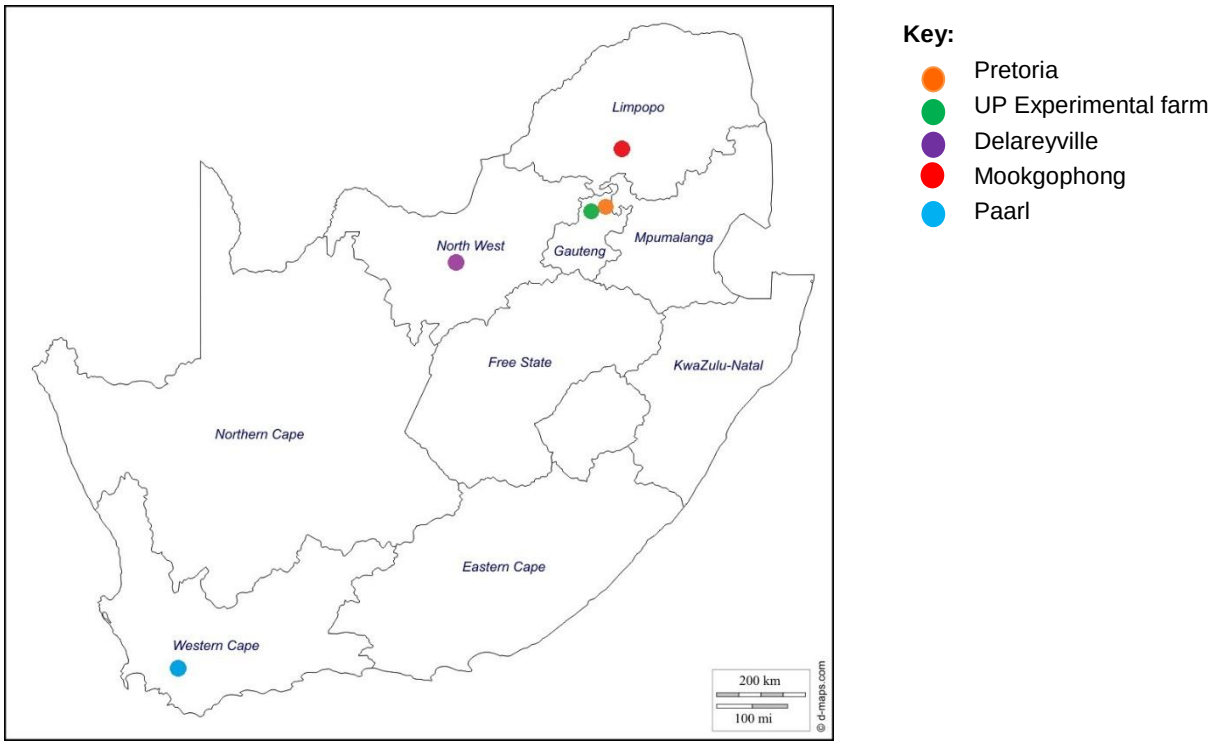

Figure 1 Map of South Africa indicating the sampling locations of goats included in this study

Ten millilitres of blood were collected from the jugular vein of each animal into EDTA tubes. The blood was kept on ice and transported to the Department of Animal and Wildlife Sciences, University of Pretoria, where it was transferred into 2-ml labelled screwtop tubes and stored at 4 °C until DNA extraction. DNA extraction was performed with a Qiagen kit (www.qiagen.com), following the standard protocol for whole blood samples.

CSN1S2 was genotyped by sequencing four gene regions to distinguish among the seven known alleles of α_{s2}-casein. Sequences were selected from the NCBI data base (www.ncbi.nlm.nih.gov) and primers were designed to sequence large DNA fragments that contain more than one allele (Table 1).

Table 1 List of primers for DNA sequencing of alpha-s_2-casein

Primer name	Alleles	Exon	Primers (5'–3')	Approximate fragment size (bp)	Final annealing temperature	NCBI Accession number
CSN1S2Ex9-11	B and D	9-11	**F** TTAATGAATTGCCCTTTCTACTCT	757	54 ˚C	AJ131465
			R CTTGTCTCGTTGGGACATTTT	653 for allele D		AJ131465
AS2-16	C and E	16	**F** TTCCCACTTAAGCATTTCAACA	346	50 ˚C	AF096872
			R GGGAGAACTCACCACATAGGG			AJ242528
CASF2	F	3	**F** GTCTCTTGCCATCAAAACAACA	310	52 ˚C	AJ238475
			R GGTCTTTATTCCTCTCTCTATA			AJ238475
EZ13	O	11	**F** GACACATAGAGAAGATTC	199	56 ˚C	AJ131465
ES11R2			**R** ATAAAAAAGCAGCACTCA			AJ131465

The polymerase chain reaction (PCR) was performed with a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems, Cheshire, UK), with a final volume of 15 µl. The amplification reaction contained 3 µl Bioline MyTaq® 5x reaction buffer (containing buffer, $MgCl_2$ and dNTPs) (www.bioline.com), 6.1 µl molecular grade water, 0.3 µl Bioline MyTaq® enzyme (www.bioline.com), 0.3 µl each of both forward and reverse primers (10 pmol/µl) and 5 µl genomic goat DNA. The PCR conditions were as follows: 94 °C for 5 min, 33 cycles of 94 °C for 45 s, final annealing temperature for 80 s and 72 °C for 1 min, with a final extension step of 72 °C for 5 min. PCR products were cleaned with ethanol precipitation to remove primers, dNTPs and other impurities.

A BigDye® Terminator v3.1 (Applied Biosystems, Cheshire, UK) reaction was used to generate sequences for each allele of *CSN1S2*. The amplification reaction contained 0.5 µl primer (10 pmol/µl), 0.8–1.5 µl BigDye (depending on DNA fragment size), 1.2–2 µl sequencing buffer (depending on the amount of BigDye), 1.5–4 µl of the cleaned PCR product (depending on DNA quantity, approximately 20–50 ng/µl, and quality after ethanol precipitation) and molecular grade water to reach a final volume of 10 µl. The PCR conditions for the cyclic sequencing reaction were as follows: 96 °C for 1 min, 25 cycles of 96 °C for 10 s, final annealing temperature for 5 s and 60 °C for 4 min. No post extension step was required. Successful amplicons were sent for automated sequencing using an ABI PRISM® 3100 Genetic Analyser (Applied Biosystems, Cheshire, UK) at the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria.

Raw sequencing data were visualised in SeqScanner as chromatograms. Further analysis was conducted using CLCBio Main workbench (www.clcbio.com) and the basic local alignment search tool (BLAST) program on the NCBI database (www.ncbi.nlm.nih.gov). Allele and genotype frequencies of *CSN1S2* were estimated by direct counting, within and between populations. Arlequin v3.5 (Excoffier & Lischer, 2010) was used to perform an analysis of molecular variance (AMOVA), to calculate pairwise F_{ST} values to determine genetic differentiation between the populations and to establish the gene diversity of the breeds, an equivalent to the expected heterozygosity of diploid data, according to Nei (1987).

Results

Allelic frequencies for α_{s2}-casein are shown in Table 2, with the A allele at the highest frequency, followed by allele F, C and B across all four populations. The rare B allele was absent in the Saanen population, while the rare D, E and O alleles of *CSN1S2* were not observed in these goat populations.

Table 2 Allele frequencies of the alpha-s_2-casein gene in South African goat populations

Allele	Saanen	British Alpine	Toggenburg	Meat goats	Dairy goat populations	Total sample population
A	0.500	0.700	0.625	0.750	0.608	0.644
B	0	0.025	0.225	0.125	0.083	0.094
C	0.200	0.050	0.075	0.050	0.108	0.094
F	0.300	0.225	0.075	0.075	0.200	0.169

Ten genotypes were detected in these South African goat populations, six genotypes being heterozygous (Table 3). The majority of individuals had the AA genotype, except for the Saanen, in which the most frequent genotype was AC. Four heterozygous genotypes were observed in the Toggenburg population, compared with two and three for the Saanen and British Alpine populations, respectively. For the meat-type goats, three heterozygous genotypes were observed, including the rare BC and CF genotypes. The rare CC genotype was observed only in the Saanen population with a relatively low frequency (0.050), whereas the BB, BC and BF genotypes occurred only in the Toggenburg dairy population (Table 3).

Table 3 Genotype frequencies of the alpha-s_2-casein gene in South African goat populations

Genotype	Saanen	British Alpine	Toggenburg	Meat goats	Dairy goat populations	Total sample population
AA	0.250	0.500	0.450	0.600	0.400	0.450
BB	0	0	0.050	0	0.017	0.013
CC	0.050	0	0	0	0.017	0.013
FF	0.200	0.100	0.050	0	0.117	0.088
AB	0	0.050	0.250	0.200	0.100	0.125
AC	0.300	0.100	0.100	0	0.167	0.125
AF	0.200	0.250	0	0.100	0.150	0.138
BC	0	0	0.050	0.050	0.017	0.025
BF	0	0	0.050	0	0.017	0.013
CF	0	0	0	0.050	0	0.013

Table 4 indicates that the largest component of the genetic variation was a result of the variation within populations (95.9%), while the remaining variation is owing to differences among populations.

Table 4 Analysis of molecular variance for the South African goat breeds

Source of variation	Sum of squares	Variance components	Percentage of variation
Among groups	0.613	-0.00479	-1.76
Among populations within groups	1.800	0.01597	5.86
Within populations	40.750	0.26122	95.90
Total	43.163	0.27240	

A fixation index (F_{ST}) of 0.041 was calculated across the populations. For the dairy breeds, the largest difference was between the Saanen and the Toggenburg (Table 5), whereas differences among all other

dairy breeds had low positive F_{ST} values ($F_{ST} < 0.05$). An unexpected small difference was observed (F_{ST} = 0.0016). between the Toggenburg and the meat-type goats The overall genetic diversity of the goat breeds was moderate, with the Saanen population having the highest genetic diversity (63.6%), followed by the Toggenburg (56.2%) and British Alpine populations (46.8%). The meat-type goats showed low to moderate genetic diversity of 42.3%.

Table 5 Population pairwise comparison of Wright's fixation index (F_{ST}) between the South African goats

	Saanen	British Alpine	Toggenburg	Meat-type goats
Saanen	0.00000			
British alpine	0.03594	0.00000		
Toggenburg	0.07890	0.04011	0.00000	
Meat-type goats	0.10527	0.01402	0.00162	0.00000

Discussion

Goat milk containing the favourable alleles of *CSN1S2* (A, B, C and F) has been shown to produce higher amounts of α_{s2}-casein, which results in milk containing significantly more total casein (Albenzio *et al*., 2009). It is associated with selection for milk yield, protein, and fat content (Vacca *et al*., 2014). In this study, A, B, C, and F alleles were observed in the South African dairy goat populations, with the A allele occurring at the highest frequency, followed by the F allele. Similar frequencies for allele A and F were reported in Italian (Vacca *et al*., 2009), Sicilian (Gigli *et al*., 2008; Palmeri *et al*., 2014), and West African goat breeds (Caroli *et al*., 2006; Caroli *et al*., 2007). The high occurrence of allele A in the South African goat population may be attributed to the A allele being considered almost fixed in the majority of Western breeds (Selvaggi & Tufarelli, 2011). It is also regarded as the ancestral variant for the α_{s2}-casein locus (*CSN1S2*) (Sacchi *et al*., 2005; Caroli *et al*., 2006). The A allele was the most frequent in the meat-type goats that were not selected for milk production.

The Toggenburg population had a frequency of 0.225 for *CSN1S2* B, which was higher than the low frequencies of between 0.005 and 0.051 reported by Caroli *et al.* (2006; 2007), Gigli *et al.* (2008) and Vacca *et al.* (2009; 2014). Furthermore, the AB genotype was observed in all the populations except for the Saanen population. The local meat-type goats also carried the B allele. Genotype combinations with the B allele (BB, BC and BF) occurred only in the Toggenburg dairy goat population at a very low frequency and were not reported in previous studies on the *CSN1S2* locus. For the meat-type goats, the BC genotype was also observed at a very low frequency of 0.050. The BB and BF genotypes of *CSN1S2* seem to be unique to the South African Toggenburg population, and may be an effect of genetic drift, in the sense that only a few rare alleles remained in this small population.

In a recent study by Vacca *et al.* (2014), the *CSN1S2* genotypes that were associated with the greatest effect on milk traits were both heterozygous (AC and CF), while the homozygous AA, CC, and FF genotypes showed no association. According to Vacca *et al.* (2014), goats with a CF genotype gave the highest daily milk yield, while goats with an AC genotype had the highest fat and protein daily production. The highest frequency for the AC genotype in the Saanen is possibly owing to this breed being the most selected among the South African dairy goat breeds. Since the majority of the dairy goats had an AA genotype, the occurrence of improved milk-production traits, based on the favourable genotypes, may be limited in the South African dairy goats. Although six of the ten genotypes observed in the total population were heterozygous, most of them occurred in the Toggenburg population with very low frequencies (0.050). These genotypes contain rare alleles in which the effect on milk production traits is largely unknown and requires further investigation, especially since these genotypes were not reported in any other goat breeds that have been studied (Palmeri *et al*., 2014; Vacca *et al*., 2009; 2014).

The rare alleles observed in the Toggenburg could be attributed to a possible population bottleneck effect, which might have resulted from the restriction on importing new breeding stock in the early 1900s. This restriction lasted for a few decades (Muller, 2005), and resulted in a small gene pool. The limited number of fixed alleles might also be due to less selection emphasis. The Saanen breed has a much larger

population size and subsequently a larger genepool of breeding animals. The British Alpine and Toggenburg breeds consist of a few small herds with low animal numbers (Muller, 2005) and breeding stock is obtained between these herds, with limited importation of new breeding animals.

Moderate genetic variation was observed among populations based on gene diversity. This was confirmed by Bosman *et al.* (2015), which indicates moderate genetic variation in South African dairy goats with a low risk of inbreeding. The low positive F_{ST} values observed in this study indicated little genetic differentiation among the three dairy goat populations, with a slightly moderate genetic differentiation between Saanen and Toggenburg. This is expected, since these three breeds have undergone production-specific selection as dairy-type animals. Little genetic differentiation was observed between the meat-type goats and the Toggenburg population (F_{ST} = 0.00162). This might be attributable to the admixture that has taken place between the Toggenburg breed and local meat-type goats in the past.

The study confirmed the presence of favourable alleles in the South African populations, indicating room for genetic improvement using directional selection for favourable genotypes. Further studies should include the quantification of the casein fractions in the milk to associate the genotypes in these populations with milk production traits.

Conclusion

The investigation of *CSN1S2* in South African dairy goats resulted in the detection of four alleles (A, B, C, and F) that have been reported to have a positive association with milk traits. The favourable AC genotype was found at a relatively high frequency in the Saanen population. The unique AB genotype, which was not reported in previous research, was detected in the Toggenburg and British Alpine dairy populations, and in the meat-type goats. Three rare genotypes were observed in the Toggenburg population (BB, BC, and BF) and two in the meat-type goats (BC and CF). The observed genetic variation in the goat populations ranged from low to moderate, indicating limited inbreeding. Future studies should focus on the quantification of casein fractions in goat milk, and the association of favourable genotypes with milk production traits.

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Authors' Contributions

For the paper entitled 'Genetic polymorphism of *CSN1S2* in South African dairy goat populations' EVM conceptualised the idea, while RG did the laboratory work and data analyses. RG, CV and EVM wrote the paper and assisted with editing the article. SC assisted with methodology development and editing the article

Conflict of Interest Declaration

None of the authors (RG, CV, SC and EMK) has a financial or personal relationship with other people or organisations that could inappropriately influence or bias this paper.

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