Polymorphism of Caprine *SLC11A1* Gene and Relationships with Hygienic Characteristics of Milk

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Summary

The solute carrier family 11 member A1 (*SLC11A1*) gene is associated with resistance to infectious diseases. Genetic variability at the 3' untranslated region (3'-UTR) of this gene is due to the presence of a polymorphic microsatellites that contain a (GT)n dinucleotide repeat. The microsatellite variability and relationships with milk yield and composition, somatic cell count (SCC) and total microbic count (TMC) were investigated in 260 goats of Sarda breed. Genotyping of the upstream guanine-thymine repeat (GT)n revealed twenty different genotypes and eight alleles (GT11, GT12, GT14, GT15, GT16, GT17, GT18 and GT19). The present study confirmed the high genetic variability of the Sarda goat and that the genotype of the microsatellite at 3'-UTR *SLC11A1* affected many chemical and hygienic characteristics of milk as fat, protein and SCC.

Key words

goat, milk, SLC11A1, hygiene

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Aim

Livestock diseases adversely affect animal productions worldwide. Susceptibility or resistance of the host is influenced by the genetic component that regulates the efficiency of the immune response to infectious diseases. Identification of candidate genes or molecular genetic markers associated with resistance traits could improve natural resistance in animal populations. One of the candidate genes studied in livestock is named *SLC11A1* (solute carrier family 11 member A1). In cattle species, some authors have studied the influence of this gene and microsatellites at 3' untranslated region (UTR) on natural resistance to brucellosis (Barthel et al., 2001) and also to the characteristics of milk hygiene, as the content of somatic cells (Zhang et al., 2009). In goat species, chromosomal localization and genetic variation of *SLC11A1* has recently been studied (Vacca et al., 2010). The aim of this research is to investigate the relationships of polymorphisms at 3'-UTR microsatellite of caprine *SLC11A1* with chemical and hygienic characteristics of milk.

Material and methods

A total of 260 goats of Sarda breed at third parturition from three different herds located in Sardinia (H1, H2 and H3) were randomly selected for this study. The animals were extensively reared, fed at pasture, supplemented with commercial food (200 g /per head/day) and hand-milked once daily in the morning. Blood samples were collected from each animal into a tube containing EDTA and DNA extracted using a commercial kit (Puregene Blood Core Kit B, Qiagen). Primer pair (forward Ex15F: 5'-GTCTGGACCTGTCTCATCACC-3' and reverse Ex15R: 5'-ACTCCCTCTCCATCTTGCTG -3') was designed on the basis of the goat genomic sequence (GenBank GU440577) to amplify a product of approximately 233 bp corresponding to a portion of the 3'-UTR (exon 15, total size 448 bp), which contains a (GT)n dinucleotide repeat. This microsatellite is also called "region A" and it is located at 5' end of exon 15, given that another microsatellite, called "region B", is also present at 3' end of exon 15 (Liandris et al., 2009). Only the polymorphism of "region A" was investigated in the present study by means of some molecular techniques. PCR was conducted using a Thermal Cycler Mastercycler® ep (Eppendorf), in a final volume of 25 µl sample containing 100 ng of genomic DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, buffer 1X (20mM Tris-HCl, pH 8.4, 50mM KCl), 0.2 mM of each primer and 1 unit of Taq Polimerase (Platinum® Taq DNA Polymerase, Invitrogen); amplification conditions for the PCR were: an initial denaturation step at 94°C for 2.5 min, 35 cycles (denaturation at 94°C for 20 s, annealing at 58°C, extension at 72°C for 30 s) and a final extension at 72°C for 10 min. PCR products were checked in 2% agarose gel (4.0 V/cm) stained with ethidium bromide (0.5 mg/ml), later purified using the Charge Switch PCR Clean-UP Kit (Invitrogen) and sequenced using an ABI Prism 3730 DNA Analyzer (Applied Biosystems). Single strand conformation polymorphism (SSCP) was later used to screen for polymorphisms of region A microsatellite. Electrophoresis was carried out on a D-CODE System for SSCP (Bio-Rad) using 8% acrylamide gels containing 1.25% glycerol in 0.5 × Tris–borate–EDTA (TBE) with a constant voltage at 15°C. Sequencing of representative samples from each pattern, obtained by SSCP analysis, evidenced that the only nucleotide differences among the goats regarded the number of (GT)n repeats.

Milk samples were collected from each goat at monthly intervals using a sterile plastic container of 50 mL. The sampling period started in March, on 45 days in milking (DIM), and finished in July, on 165 DIM. On the same day of sample collection, daily milk yield was recorded for each animal. Samples were stored at 4°C and transported to the laboratory within two hours. All the samples were analyzed for: fat, total protein and pH by an infrared spectrophotometer (Milko–Scan 133B; Foss Electric, DK–3400 Hillerød, Denmark) according to International Dairy Federation standards (IDF 141C:2000); somatic cells count (SCC) using an automatic cell counter (Fossomatic 90, Foss Electric) according with IDF 148A:1995 standards; total microbial count (TMC) using a Bacto-Scan, Foss Electric (IDF 358:2000).

Data were subjected to statistical analysis, using Minitab Release 13.32 software (Minitab Inc. 2000, State College, PA) and a repeated measures General Linear Model procedure (GLM), according to the model:

$$Y_{ijk} = \mu + H_i + M_j + G_k + e_{l(ijk)}$$

where Y_{ijk} is the analyzed parameter, μ is the mean, H_i is the random effect of herd (i = 3), M_j is the fixed effect of month of sampling (j = 5), G_k is the random effect of microsatellite genotype at 3'-UTR of *SLC11A1* (k=7) and $e_{l(ijk)}$ is the error effect. Only genotypes with frequency higher than 3.46% were included in the statistical model, for a total of 227 goats. Before statistical analysis, SCC and TMC were transformed into logarithmic form to normalize their frequencies. Model effects were declared significant at $P<0.05$.

Results and discussion

SSCP analysis evidenced 20 different polymorphic patterns. All the animals were grouped on the basis of their own SSCP banding profiles, the amplified fragments had a size between 222 and 238 bp and the number of GT repeats ranged from 11 to 19. A total of eight alleles were identified (GT11, GT12, GT14, GT15, GT16, GT17, GT18 and GT19), while the GT13 allele was not detected.

The distribution of genotypes at 3'-UTR microsatellite is illustrated in Table 1. The highest frequency was recorded for GT16/GT16, which had a frequency of 38.85% in the overall population and was present in all the three herds, and the most frequent heterozygous genotype was GT16/GT19 (14.62%).

Allele frequency at 3'-UTR microsatellite is illustrated in Table 2. The most frequent allele was GT16 (62.5%), followed by GT19 (10.96%), GT14 (10.77%) and GT17 (6.92%). It is remarkable the absence in all the three herds of allele GT13, which in some cattle breeds represents 100% of the population (Paixão et al., 2006; Pazzola et al., 2009) and has been associated with resistance to brucellosis (Barthel et al., 2001). We can speculate that this allele is not favourable for goat species because it causes a reduced fitness.

Some authors have studied both microsatellites (region A and B) in native Greek goats and the association of their polymorphisms with innate resistance to paratuberculosis; two different alleles have been identified (GT7 and GT8) in region B that are associated with resistance, whereas any of four alleles

Table 1. Genotype frequencies at the 3 '-UTR *SLC11A1* microsatellite

Genotype	Herd						Total population	
	H1		H2		H3			
	n	%	n	%	n	%	n	%
GT11/GT15	2	2.41	-	-	-	-	2	0.77
GT11/GT16	4	4.82	-	-	-	-	4	1.54
GT12/GT14	-	-	1	1.27	4	4.08	5	1.92
GT12/GT16	2	2.41	5	6.33	3	3.06	10	3.85
GT12/GT19	-	-	1	1.27	3	3.06	4	1.54
GT14/GT14	-	-	-	-	2	2.04	2	0.77
GT14/GT15	1	1.20	-	-	-	-	1	0.38
GT14/GT16	6	7.23	4	5.06	25	25.51	35	13.46
GT14/GT17	-	-	-	-	2	2.04	2	0.77
GT14/GT19	2	2.41	-	-	7	7.14	9	3.46
GT15/GT16	10	12.05	-	-	-	-	10	3.85
GT15/GT17	3	3.61	-	-	-	-	3	1.15
GT15/GT19	2	2.41	-	-	-	-	2	0.77
GT16/GT16	31	37.35	50	63.29	20	20.41	101	38.85
GT16/GT17	11	13.25	3	3.80	10	10.20	24	9.23
GT16/GT18	2	2.41	-	-	-	-	2	0.77
GT16/GT19	7	8.43	13	16.46	18	18.37	38	14.62
GT17/GT17	-	-	1	1.27	1	1.02	2	0.77
GT17/GT19	-	-	1	1.27	2	2.04	3	1.15
GT18/GT19	-	-	-	-	1	1.02	1	0.38
Total	83	100	79	100	98	100	260	100

Table 2. Allele frequencies at the 3 '-UTR *SLC11A1* microsatellite

Allele	Herd			Total population (%)
	H1 (%)	H2 (%)	H3 (%)	
GT11	3.61	-	-	1.15
GT12	1.20	4.43	5.10	3.65
GT14	5.42	3.16	21.43	10.77
GT15	10.84	-	-	3.46
GT16	62.65	79.11	48.98	62.50
GT17	8.43	3.80	8.16	6.92
GT18	1.20	-	0.51	0.58
GT19	6.63	9.49	15.82	10.96

(GT14, 15, 16 or 18) in region A microsatellite are not associated with resistance (Liandris et al., 2009). In addition to those four alleles of region A identified by Liandris et al. (2009), in the present study four novel alleles regarding region A (GT11, 12, 17, 19) were recognized in the Sarda goat.

The results of milk traits according the herd, the stage of lactation and the polymorphism at microsatellite 3'-UTR of SLC11A1 gene are shown in Table 3 and Table 4. These results are probably biased by the large number of false positive effects deriving from the statistical analysis, because it was assumed that the genetic effects were randomly distributed and the absence of a variance-covariance structure between the measurements in the different stages of lactation.

The highest mean of milk yield was recorded in H3 and on 75 and 105 DIM. Fat and protein were higher in H2. Heterozygous animals GT15/GT16 had the highest values of milk production

Table 3. Means and standard error (se) of milk yield and composition according to the herd, stage of lactation and genotype at microsatellite 3'-UTR of *SLC11A1* gene

			Milk yield		Fat		Protein	
		(n)	(g/day)	se	(%)	se	(%)	se
Herd	H1	(345)	933^{B}		4.32^{C}		3.90^{C}	
	H2	(375)	940^{B}		4.99^{A}		4.15^{A}	
	H3	(415)	1050^{A}	13	4.84^{B}	0.03	4.03^{B}	0.02
Stage of lactation (DIM)	45	(227)	1005^{Q}		4.42^{R}		4.18^{P}	
	75	(227)	1138^{P}		4.77^{Q}		4.15^{PQ}	
	105	(227)	1138^{P}		4.57^{QR}		4.03^{Q}	
	135	(227)	793^{R}		5.06^{P}		3.93^{R}	
	165	(227)	798^{R}	12	4.77^{Q}	0.03	3.84^{R}	0.02
Genotype	GT12/GT16	(50)	955^{wx}		4.85^{W}		4.20^{W}	
	GT14/GT16	(175)	995^{w}		4.62^{W}		3.96^{X}	
	GT14/GT19	(45)	925^{wx}		4.52^{WX}		4.08^{WX}	
	GT15/GT16	(50)	1134^{w}		4.44^{X}		3.72^{Y}	
	GT16/GT16	(505)	949^{x}		4.83^{W}		4.07^{W}	
	GT16/GT17	(120)	897^{x}		5.07^{W}		4.19^{W}	
	GT16/GT19	(190)	964^{wx}	13	4.69^{W}	0.03	3.96^{X}	0.02
Effect and significance	H		***		***		***	
	DIM		***		***		***	
	G		*		***		***	

H: herd; DIM: days in milking; G: genotype. ^{ABC} Differ at P<0.001 in herd comparison; ^{PQR} differ at P<0.001 in DIM comparison; ^{WXY} differ at P<0.001 in genotype comparison. ^{wxy} differ at P<0.05 in genotype comparison. Significance levels: * = P<0.05; *** = P<0.001

Table 4. Means and standard error (se) of pH, somatic cells count (SCC) and total microbial count (TMC) of milk according to the herd, stage of lactation and genotype at microsatellite 3'-UTR of *SLC11A1* gene

			pH		SCC		TMC	
		(n)		se	(log/mL)	se	(log/mL)	se
Herd	H1	(345)	6.70^{A}		6.28^{A}		4.93^{A}	
	H2	(375)	6.61^{C}		5.97^{B}		4.44^{B}	
	H3	(415)	6.66^{B}	0.01	5.93^{B}	0.02	4.40^{B}	0.04
Stage of Lactation (DIM)	45	(227)	6.67^{P}		5.79^{R}		4.09^{S}	
	75	(227)	6.66^{P}		5.94^{Q}		4.34^{R}	
	105	(227)	6.65^{P}		5.95^{Q}		4.51^{R}	
	135	(227)	6.67^{P}		6.31^{P}		5.15^{P}	
	165	(227)	6.63^{Q}	0.01	6.29^{P}	0.02	4.87^{Q}	0.04
Genotype	GT12/GT16	(50)	6.67^{XY}		5.88^{x}		4.49	
	GT14/GT16	(175)	6.65^{XY}		6.15^{w}		4.69	
	GT14/GT19	(45)	6.63^{XY}		6.17^{wx}		4.84	
	GT15/GT16	(50)	6.71^{W}		5.94^{w}		4.33	
	GT16/GT16	(505)	6.65^{Y}		6.08^{w}		4.57	
	GT16/GT17	(120)	6.65^{X}		6.06^{w}		4.60	
	GT16/GT19	(190)	6.65^{XY}	0.01	6.12^{w}	0.02	4.62	0.04
Effect and significance	H		***		***		***	
	DIM		***		***		***	
	G		***		*		NS	

H: herd; DIM: days in milking; G: genotype. ^{ABC} Differ at P<0.001 in herd comparison; ^{PQRS} differ at P<0.001 in DIM comparison; ^{WXY} Differ at P<0.001 in genotype comparison. ^{wx} differ at P<0.05 in genotype comparison. Significance levels: NS = not significant; * = P<0.05; *** = P<0.001

but lower levels in fat and protein content. On the whole data regarding milk yield and composition are similar than those published for multiparous Sarda goats by Vacca et al. (2006) and

Morand-Fehr et al. (2007). The pH was significantly affected by all the three factors, and GT16/GT17 had the lowest mean value; checking of this parameter is useful because it is considered as a marker for subclinical mastitis and deeply affect coagulation properties of milk (Pirisi et al., 2007). SCC was affected by herd and stage of lactation at P<0.001 and genotype at P<0.05; the highest mean SCC value was recorded in H1. The logarithmic values were close to the value of 6, which corresponds to 1.000.000 cells/ml.

Nowadays, in the United States these values could cause the unsuitability of milk for human consumption, in accordance with local laws (US PMO, 2007). On the contrary, in the European Union there is not any threshold limit values for SCC in goat milk (EU, 2004). The lowest mean of SCC value was recorded for genotype GT12/GT16. This data is statistically significant, but has a limited implication in the goat, because the value of SCC in this species is also correlated with several factors other than udder health. Raynal–Ljutovac et al. (2007) state that stage of lactation is the most important non-infectious factor which affects SCC. SCC in goat milk is higher if compared to other dairy species, but several studies clarified that the physiological processes of mammary secretion, based on an apocrine process, cause a high number of somatic cells in milk (Park and Humprey, 1986). TMC was affected by the herd and stage of lactation (P<0.001) but it was not affected by genotype. Mean values of TMC were satisfactory, considering that the present research was conducted in traditional farms and goats were hand milked. Indeed, mean values of TMC were almost constantly lower than 500.000 bacteria/ml, in compliance with the limits fixed by EU Regulation 853/2004 (EU, 2004).

Conclusions

The genotype of *SLC11A1* 3'-UTR microsatellite in the present study affected many chemical and hygienic characteristics of milk. Milk yield, fat and protein, which are commonly measured for milk production controls of the Italian National Association of Breeders (2008), were significantly influenced by genotype. Among the traits regarding hygienic characteristics of milk, pH and SCC were affected by the genotype, but not TMC. As regards SCC, a high number of somatic cells and particles of the mammary secretory epithelium are counted by the most common methods in dairy laboratories (Haenlein, 2001) and, at present time, to solve this problem new methods as flow cytometry and microscopy are proposed to discriminate inflammatory cells in goat milk (Boulaaba et al., 2011). Total microbial count is an important parameter commonly utilized to evaluate hygienic characteristics of milk (UE, 2004), but given that *SLC11A1* gene is associated with resistance to intracellular pathogens, further studies that are able to count intracellular bacteria of TMC are required.

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