PCR-SSCP analysis of GH gene in Sarda goats: a high variability and its preliminary effects on dairy performances

Maria Luisa Dettori, Angela Maria Rocchigiani, Michele Pazzola, Vincenzo Carcangiu, Giuseppe Massimo Vacca

Dipartimento di Biologia Animale, Università di Sassari, Italy

Corresponding author: Maria Luisa Dettori. Dipartimento di Biologia Animale, Università di Sassari. Via Vienna 2, 07100 Sassari, Italy - Tel. +39 079 229435 - Fax: +39 079 229592 - Email: mldettori@uniss.it

ABSTRACT - The growth hormone (GH) gene can be utilized as a major gene because in various domestic livestock its polymorphisms have been associated to milk traits. The aim of this research was to investigate single-strand conformation polymorphism (SSCP) in the exon 3 of gGH (goat GH) gene and to evaluate the possible association with milk traits in Sarda goat breed. Forty-four primiparous lactating goats were randomly chosen, and the productive parameters (milk yield, fat, protein, and lactose percentage) of three consecutive lactations were monitored. The exon 3 of the gGH gene was PCR amplified and the resulting products were analysed by SSCP. Six conformational patterns were detected. The sequencing of SSCP patterns revealed the occurrence of six nucleotide changes, two of which determined amino acid changes in the deduced protein sequence. A preliminary comparative analysis of the productive traits related to three lactations with the genomic profiles derived from the SSCP analysis was performed with the ANOVA statistical method. SSCP polymorphic patterns in exon 3 were associated ($P<0.01$) with milk yield, fat and protein percentages, and with lactose content ($P<0.05$). These findings may be used for marker assisted selection in Sarda goat, in order to improve dairy production, preserving genetic diversity of the population.

Key words: Goat growth hormone, Milk traits, SSCP polymorphism.

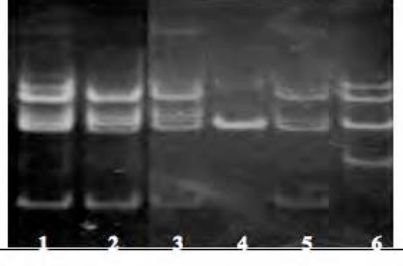
Introduction - Growth hormone (GH) plays a key role in the control of metabolic and physiological processes. GH is an anabolic hormone synthesized by the somatotroph cells of the anterior lobe of the pituitary gland influencing processes such as growth rate, lactation, as well as protein, lipid, and carbohydrate metabolism (ThidarMyint *et al.*, 2008). For this reason, its possible utilization as a candidate gene marker to improve production in farm animals raises great interest (Min *et al.*, 2004). The GH gene spans 2.6 to 3.0 kbp in most mammalians and comprises five exons and four introns. Genetic polymorphisms at GH gene have been detected in cattle revealing a close relationship with milk traits. Some of these polymorphisms were significantly associated with fat (Falaki *et al.*, 1996) or protein percentage (Lagziel *et al.*, 1999). A PCR-RFLP polymorphism was identified in the intron 3 of bGH (bovine GH) gene, which appeared to be associated with milk yield and milk fat and protein content (Zhou *et al.*, 2005). Association between oGH (ovine GH) gene genotypes and milk yield was found also in sheep (Marques *et al.*, 2006). SNPs were found in gGH (goat GH) gene exon 4 and exon 5 in a prolific meat breed of India, the black Bengal goat (Gupta *et al.*, 2007). Associations were established in the Portuguese Algarvia goat breed between gGH SSCP polymorphic patterns in exon 4 and 5 and milk production (Malveiro *et al.*, 2001), later confirmed by Marques *et al.* (2003) for the Serrana goat. None of these authors found any association of polymorphisms of exon 3 with milk traits. The Sarda goat is well adapted to its natural environment, but its preservation is threatened and it may be defended by a genetic selection aiming to improve productive traits. The objective of this research was to investigate single-strand conformation polymorphisms in the exon 3 of gGH gene and to establish the relationship between these polymorphisms and milk production traits in the Sarda goat breed.

Material and methods - The research was carried out on 44 Sarda goats belonging to a typical farm located in Sardinia. Primiparous lactating goats were randomly chosen, and the productive parameters related to three consecutive lactations were monitored. During each lactation, at 45, 75, 105, 135, and 165 days after parturition, the daily milk yield was recorded and a milk sample was collected to determine fat, protein, and lactose content, using an I.R. spectrophotometer (Milko-Scan, Foss Electric). A blood sample was collected from each animal and genomic DNA was extracted using a Puregene DNA Isolation Kit (Gentra). A 157-bp-long fragment containing the exon 3 of the gGH gene was amplified by PCR. The utilized primer pair and PCR conditions were as described by Marques *et al.* (2003). The SSCP analysis was carried out on a D-Code Universal Mutation Detection System (BioRad), as follows: 2.5 µL of PCR product was added to 7.5 µL of denaturating solution (1mg/ml xylene-cyanol, 1mg/ml bromophenol blue, and 10mM EDTA in 80% deionized formamide). After denaturation at 94°C for 3 min, the samples were rapidly chilled on ice and then run (7.30 h, 25 W, 15°C) on 12% acrylamide:bisacrylamide gels (37.5:1) in 1× Tris-borate-EDTA (TBE) buffer. The DNA fragments showing different patterns on SSCP gels were sequenced. Analysis of nucleotide sequences and deduced amino acid sequences was performed with BioEdit (www.mbio.ncsu.edu/BioEdit/) software. In order to assess the effects of the profiles derived from gGH SSCP on milk traits, genomic data and all records of the three lactations were submitted to one way analysis of variance and Tukey multiple comparison test.

Results and conclusions - The PCR-SSCP analysis of the DNA fragment containing the exon 3 of the gGH gene revealed six different polymorphic patterns, numbered I to VI. Generally, two single strand DNAs should produce only two conformation polymorphisms, and yield two SSCP bands in homozygosis, four bands in heterozygosis. This is the case of lane 4 and lane 6 (Figure 1A). However, some electrophoretic profiles showed six (lane 3) to seven (lane 1) bands. This might be due to the presence of two stable conformations for one of the single strands (Rubio *et al.*, 1996). Another explanation to this variability may be the presence of more than one copy of the gGH gene, a type of genomic modification defined "Copy Number Variation" (CNV), which has been documented, at this locus, in the ovine and caprine species (Yamano *et al.*, 1991). Indeed, each animal can have two to four gGH genes (for diploid genome), depending on the CNV allele: the gGH1 allele contains only one copy of the gGH gene, while the gGH2 and gGH3 alleles are duplicated in tandem, which complicates interpretation of SSCP banding patterns.

Figure 1. A) PCR-SSCP results for gGH locus; B) Nucleotide and amino acid changes found.

A)



Lane	SSCP profile
1	III
2	VI
3	IV
4	I
5	V
6	II

B)

nt at exon 3	nt variation	aa variation
42	G_A	Gln
69	C_T	Ser
81	G_A	Pro
91	G_A	Gly_Ser
99	T_C	Asn
100	G_C	Glu_Gly

Sequencing of SSCP patterns of gGH gene exon 3 revealed the occurrence of six nucleotide changes (Figure 1B) in comparison to the sequence published under Acc. no. D00476. Among these mutations, there were five transitions and one transversion. Sequencing of pattern I showed that it corresponded to the cited published sequence (NCBI Acc. no. DD00476). Two of the detected mutations resulted in the substitution of residues in the deduced amino acid sequence: the transition G→A at nt 91, which occurred in the profiles II

Table 1. Number of animals and mean value of milk traits associated with SSCP patterns.

	subject	records	Milk yield (g/d)		Fat (%)		Protein (%)		Lactose (%)	
Pattern	n.	n.	M	SD	M	SD	M	SD	M	SD
I	20	300	755.0^{B}	361.9	5.21^{A}	1.28	4.37^{AB}	0.56	4.80^{b}	0.49
II	6	90	869.0^{BC}	478.7	5.51^{AB}	0.87	4.38^{AB}	0.40	4.89^{b}	0.44
III	8	120	947.7^{C}	474.2	5.15^{A}	0.92	4.15^{A}	0.42	4.97^{b}	0.43
IV	4	60	781.7^{BC}	320.2	5.77^{B}	0.77	4.52^{B}	1.06	4.47^{a}	0.78
V	2	30	717.1^{AB}	239.3	6.76^{C}	1.02	5.25^{C}	0.85	4.79^{b}	0.30
VI	4	60	517.8^{A}	229.4	5.85^{B}	0.87	4.40^{AB}	0.35	4.84^{b}	0.46

Capital letters are significantly different for P<0.01, lower-case letter for P<0.05.

and VI, and the transversion G→C at nt 100, appeared only in the II profile. Table 1 shows, for each SSCP pattern, the number of goats carrying the patterns and mean values of milk traits.

The most represented profile was the I and the rarest was the V. Statistical analysis showed that animals with SSCP pattern III had a higher milk yield, while pattern V was associated with a higher fat and protein percentage (P<0.01). Our results suggest that high genetic variability exists at the GH gene locus in Sarda goat, as it was seen in the Algarvia and Serrana goat. Furthermore, polymorphic patterns at exon 3 were positively associated with milk production, and with both fat and protein content. These results are preliminary, and they should be confirmed on a larger sample size. The gGH gene polymorphisms may be used for marker assisted selection in Sarda goat, also taking into account short and long-term effects on population structure and rates of inbreeding, in order to improve dairy production preserving genetic diversity of the population.

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