SHORT COMMUNICATION

A new polymorphism in goat \(\beta\)-lactoglobulin promoter region

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ABSTRACT

An individual variability in \(\beta\)-lactoglobulin content has been previously observed in Girgentana goat milk by HPLC analysis. To identify eventual mutations affecting the transcription level of the gene, the promoter region was characterized in goats showing an anomalous phenotype, consisting in a reduced content of \(\beta\)-lactoglobulin respect to \(\alpha\)-lactoalbumine. A single nucleotide substitution not previously reported has been detected. A PCR-RFLP procedure was developed for fast detection of the mutation in different goat breeds: Girgentana, Garganica, Sarda, Alpine, Montefalcone and Saanen. The Montefalcone goat showed the highest frequency of the mutation, confirming once more the peculiarity of this breed.

Key words: \(\beta\)-lactoglobulin, Goat, Promoter region, Genetic polymorphism.

RIASSUNTO

UN NUOVO POLIMORFSIMO NEL PROMOTORE DELLA \(\beta\)-LATTGOLOBULINA CAPRINA

Una variabilità individuale nel contenuto di \(\beta\)-lattoglobulina è stata precedentemente osservata nel latte di capra Girgentana mediante analisi HPLC. Per identificare eventuali mutazioni che influiscano sul livello di trascrizione del gene, la regione del promotore è stata caratterizzata nelle capre con fenotipo anomalo, cioè con un contenuto di \(\beta\)-lattoglobulina inferiore a quello di \(\alpha\)-lattoalbumina. È stata identificata una transizione T\(\rightarrow\)C in posizione -341 della regione del promotore. Una procedura PCR-RFLP è stata sviluppata per rivelare rapidamente la mutazione in differenti razze caprine: Girgentana, Garganica, Sarda, Alpine, Montefalcone e Saanen. La razza di Montefalcone presenta la più alta frequenza della mutazione pari a 0,46, confermando ancora una volta la peculiarietà di questo tipo genetico autoctono.

Parole chiave: \(\beta\)-lattoglobulina, Capra, Promotore, Polimorfismo genetico.

Introduction

\(\beta\)-lactoglobulin is a globular protein belonging to the lipocalin family. It is one of the major whey proteins present in the milk of ruminants (Mercier and Villette, 1993) and other species like pigs (Alexander and Beattie, 1992), horses (Godovac-Zimmermann et al., 1985), and donkeys (Godovac-Zimmermann et al., 1990). The cDNA encoding for \(\beta\)-lactoglobulin has been sequenced in several species, among them:
cows (Alexander et al., 1989), sheep (Gaye et al., 1986) and goats (Folch et al., 1993). The nucleotide sequences of these cDNAs are quite well conserved, especially in the coding region.

To date, several variants of bovine and ovine β-lactoglobulin have been described at DNA level (Eigel et al., 1984; Gaye et al., 1986; Erhardt, 1989). Bovine β-lactoglobulin polymorphism has been associated with milk production traits and cheese yield (Ng-kwai-Hang and Grosclaude, 1992). In sheep, several studies have investigated the influence of the variants on milk composition and technological properties, but without a clearly definite conclusion (Lopez-Galvez et al., 1995, Recio et al., 1995, Pietrolà et al., 2000). In goats, some variations at phenotypic level have been detected (Macha, 1970; Moioli et al., 1998), but only two genetic variants were described (Pena et al., 2000).

Mutations on the promoter region are those most likely responsible for different levels of gene expression. Sequence analysis of bovine β-lactoglobulin promoter showed polymorphisms associated with milk protein variants (Wagner et al., 1994, Lum et al. 1997).

The β-lactoglobulin promoter region has also been characterized in sheep (Watson et al., 1991) and the recognition motifs for several transcription factors are also conserved in goats (Folch et al., 1994). A polymorphism in the proximal promoter region has recently been reported (Yahyaoui et al. 2000) in Spanish and French goats.

In a previous work (Chianese et al. 2000) differences in β-lactoglobulin content have been detected in the milk of the Italian Girgentana goats. In this work, the promoter region was characterized in individuals of this breed showing a reduced content of β-lactoglobulin. Additional breeds were then analyzed for the polymorphism found.

**Material and methods**

DNA was isolated from blood following standard protocols. Samples from 196 animals of different Italian and French breeds were analyzed.

A fragment of 555 bp in the most proximal promoter region (-419 to +136), in which there is a high probability that several transcription factors bind, was amplified using primers designed according to the β-lactoglobulin goat sequence.

![Figure 1. Genotyping, by PCR-RFLP, of the polymorphism found in the goat β-lactoglobulin promoter region.](image-url)

Lane 1 and 6: φX174 marker.
Lane 2: undigested PCR product.
Lane 3: homozygous T/T.
Lane 4: heterozygous T/C
Lane 5: homozygous C/C
available on database (EMBL Z33881) and located in the proximal promoter region (-419 to -397) and in the exon 1 (+114 to +136), respectively.

Forward primer: 5' TCA CAG AGA TCC CTT CAC CC 3'
Reverse primer: 5' CTT GAT GTC CAG GCC TTT CA 3'

The PCR was performed in a 25 µl reaction mixture consisting of 100 ng genomic DNA, 2.5 U of Taq DNA polymerase (Promega, Madison, WI), 2 mM MgCl2, 200 µM of each dNTP, 0.4 µM of each primer, 1X PCR buffer. The thermal cycling conditions, according to Yahyaoui et al. (2000), were: a denaturation step of 95°C for 5 min, 10 cycles of 97°C for 15 s, 63°C for 1 min and 72°C for 1 min 30 sec, followed by 25 cycles of 95°C for 30 s, 63°C for 1 min and 72°C for 1 min 30 sec, with a final extension of 72°C for 10 min, using a GeneAmp PCR System 9700 (Applied Biosystems, CA).

The PCR products were purified by Microcon (Millipore Corporation, MA) and sequenced using the Big Dye Terminator DNA Sequencing kit on an ABI PRISM 310 automated sequencer (Applied Biosystems, CA). For the PCR-RFLP protocol developed for rapid detection of the mutation, the polymorphism affects the Bfa I target site, so that digestion of the PCR product from the Wild Type (WT) generates three fragments (328 + 147 + 80 bp), while Mutant (M) produces two fragments (328 + 227 bp) (Figure 1).

A total of 196 genomic DNA samples from animals of Italian (Girgentana, Garganica, Montefalcone and Sarda) and cosmopolitan (Alpine and Saanen) breeds were genotyped using the developed test. The genotypic frequencies are shown in Table 1.

Results and discussion

The promoter region of Girgentana goats was analyzed. A fragment of 555 bp was successfully amplified and sequenced.

The comparison of the obtained sequences to the one available on database (EMBL Z33881) showed the presence of a mutation at position -341 of the goat β-lactoglobulin promoter region (EMBL, AJ292058). The polymorphic site consists of a transition T→C.

A Bfa I PCR-RFLP protocol was developed for rapid detection of the mutation. The polymorphism affects the Bfa I target site, so that digestion of the PCR product from the Wild Type (WT) generates three fragments (328 + 147 + 80 bp), while Mutant (M) produces two fragments (328 + 227 bp) (Figure 1).

A total of 196 genomic DNA samples from animals of Italian (Girgentana, Garganica, Montefalcone and Sarda) and cosmopolitan (Alpine and Saanen) breeds were genotyped using the developed test. The genotypic frequencies are shown in Table 1.

All the breeds resulted in Hardy-Weinberg equilibrium, calculated using the χ² method, for the alleles present at -341 nucleotide. The Montefalcone goat showed a very high frequency of the mutation. This result once more confirms the peculiarity of this breed, as reported in the case of other milk proteins (Bevilacqua et al. 2001, Angiolillo et al. 2002). The polymorphism is also quite diffused in the Saanen goat.

The breeds used in the study have also been analyzed to investigate the eventual presence of the promoter polymorphism described by Yahyaoui et al. (2000) at position -60. This mutation has been found in all the populations examined, with a higher frequency in the Saanen breed, followed by the Sarda and by all the others (Table 1).

Table 1. Variation of the genotypic frequencies at the -341C and -60T loci of the goat β-lactoglobulin promoter region in different breeds.

| Breed        | Animals n. | -341 T/T | -341 C/T | -341 C/C | -60 C/C | -60 T/T |
|--------------|------------|----------|----------|----------|---------|---------|
| Girgentana   | 51         | 0.69     | 0.27     | 0.04     | 0.94    | 0.06    | 0       |
| Garganica    | 32         | 0.88     | 0.09     | 0.03     | 0.94    | 0.06    | 0       |
| Montefalcone | 28         | 0.29     | 0.5      | 0.21     | 0.96    | 0.04    | 0       |
| Sarda        | 35         | 0.83     | 0.17     | 0        | 0.74    | 0.26    | 0       |
| Alpine       | 19         | 0.79     | 0.21     | 0        | 0.95    | 0.05    | 0       |
| Saanen       | 31         | 0.49     | 0.44     | 0.07     | 0.61    | 0.39    | 0       |
| Total        | 196        |          |          |          |         |         |         |
Combination of the two polymorphic sites resulted in three of the possible haplotypes since the combination C (-341)/T (-60) was never observed at homozygous state.

The polymorphism is also present in the Girgentana goats that do not show anomalous phenotype. The likely hypothesis is that this new polymorphism is not the only factor responsible for the reduced content of $\beta$-lactoglobulin.

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REFERENCES

Alexander, L.J., Beattie, C.W. 1992. Sequence of porcine $\beta$-lactoglobulin cDNA. Anim. Genetics. 23:263-265.

Alexander, L.J., Hayes, G., Pearse, M.J., Beattie, C.W., Stewart, A.F., Willis, I.M. Mackinlay, A.G., 1989. Complete sequence of the bovine $\beta$-lactoglobulin cDNA. Nucleic Acids Res. 17:6739.

Angiolillo, A., Yahiyoumi, M.H., Sanchez, A., Pilla, F., Folch, J.M. 2002 Characterisation of a New Genetic Variant in the Caprine k-casein Gene. J. Dairy Sci. 85:2679-2680.

Bevilacqua, C., Ferranti, P., Garro, G., Veltri, C., Lagonigro, R., Lerboux, C., Pietrola, E., Addeo, F., Pilla, F., Chianese L., Martin, P. 2001 Interallelic recombination is likely responsible for the occurrence of a new rare cas1-casein variant in the goat species. Eur. J. Biochem. 269:1293-1303.

Chianese, L., Portolano, B., Troccone, E., Pizzolongo, F., Ferranti, P., Addeo, F., Alcata, M.L., Pilla, F., Calagna, G. 2000. The quality of Girgentana goat milk. Proc. 7th Int. Conf. on Goats, Tours, France, 2:946-949.

Erhardt, G. 1989. Evidence for a third allele at the $\beta$-lactoglobulin locus of sheep milk and its occurrence in different breeds. Animal Genetics, 20:197-207.

Eigel, W.N., Butler, J.P., Ernstrom, C.A., Farrel, H.M., Harwalkar, V.R., Jenness, R., Whitney, R.M. 1984. Nomenclature of protein of cow’s milk: fifth revision. J. Dairy Sci. 67:1599-1631.

Folch, J.M., Coll, A., Sanchez, A. 1993. Cloning and sequencing of the cDNA encoding goat $\beta$-lactoglobulin. J. Anim. Sci. 71:2832.

Folch, J.M., Coll, A., Sanchez, A. 1994. Complete sequence of the caprine $\beta$-lactoglobulin gene. J. Dairy Sci. 77:3493-3497.

Gaye, P., Hue-Delhaie, D., Mercier, J.C., Soulier, S., Virotte, J.L., Furet, J.P. 1986. Ovine $\beta$-lactoglobulin messenger RNA: nucleotide sequence and mRNA levels during functional differentiation of the mammary gland. Biochimie. 67:1097-1107.

Goddovac-Zimmermann, J., Conti, A., Liberatori, J., Braunitger, G., 1985 The amino-acid sequence of $\beta$-lactoglobulin II from horse colostrum (Equus caballus, Perissodactyla): $\beta$-globulins are retinol-binding proteins. Biol. Chem. Hoppe Seyler. 366:601-608.

Goddovac-Zimmermann, J., Conti, A., Sheil, M., Napolitano, L., 1990. Covalent structure of the minor monomorphic $\beta$-lactoglobulin II component from donkey milk. Biol. Chem. Hoppe Seyler. 371:871-879.

Lopez-Galvez, G., Juarez, M., Ramos, M. 1995. Two dimensional electrophoresis and immunoblotting for the study of ovine whey protein polymorphism. J. Dairy Res. 62:311-320.

Luc, L.S., Dovc, P., Medro, J. 1997. Polymorphism of bovine $\beta$-lactoglobulin promoter and differences in the binding affinity of activator protein-2 transcription factor. J. Dairy Sci. 80:1389-1397.

Macha, J. 1970. Protein polymorphism in goat’s milk. Zivocinisa Vyroba. 15:801-805.

Merceur, J.C., Virotte, J.L. 1993. Structure and function of milk protein genes. J. Dairy Sci. 76:3079-3098.

Moholl, B., Pilla, F., Tripaldi, C. 1998. Detection of milk protein genetic polymorphisms in order to improve dairy traits in sheep and goats: a review. Small Ruminant Research. 27:185-195.

Ng-Kway-Hang, K.G., Grosclaude, F. 1992. Genetic polymorphism of milk proteins. In: F.F. Fox (ed.) Advanced Dairy Chemistry. Elsevier Science Publishers Ltd., London and New York, pp 406-456.

Pena, R.N., Sanchez, A., Folch, J.M. 2000. Characterization of genetic polymorphism in the goat beta-lactoglobulin gene. J. Dairy Res. 67:217-214.

Pietrola, E., Carta, A., Franchi, A., Pareda, G., Pilla, F. 2000. Effect of $\beta$-lactoglobulin locus on milk yield in Sarda ewes. Zoot. Nutri. Anim. 26:131-135.

Recio, I., Molina, E., Ramos, M., de Frutos, M. 1995. Quantitative analysis of major whey proteins by capillary electrophoresis using uncoated capillaries. Electrophoresis. 16:654-658.

Yahiyoumi, M.H., Pena, R.N., Sanchez, A., Folch, J.M. 2000. Polymorphism in the goat $\beta$-lactoglobulin proximal promoter region. J. Animal Sci. 78:1100-1101.

Wagner, V., Schild, T.A., Geldermann, H. 1994. Application of polymorphic DNA sequences to differentiate the origin of decomposed bovine meat. Forensic Sci. Int. 64:89-95.

Watson, J.C., Gordon, K.E., Robertson, M., Clark, A.J., 1991. Interaction of DNA-binding proteins with a milk protein gene promoter: in vitro identification of a mammary gland-specific factor. Nucleic Acid Research. 19:6603-6610.