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Cloning and comparative analysis of gene structure in promoter site of alpha-s1 casein gene in Naeinian goat and sheep

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ABSTRACT

The 5′ end or alpha-S1 casein promoter has a significant role in milk protein gene expression. The understanding of the translation process of alpha-S1 casein mutants will provide us an opportunity to make the best selection in livestock providing more proteins in milk. Blood samples were taken from three hundred of Naeinian goats and sheep, and DNA extraction was done using modified salting out method. Polymerase chain reactions (PCR) were carried out using a specific primer pairs for amplification a fragment of 1133 bp from part of 5′-UTR and exon 1 of alpha s1 casein gene. The *Alu*I and *Hin*fI restriction enzyme treatment of all samples provided the same homozygous AA genotype in both species. Subsequently, one sample of each species was selected and cloned, and the final sequences were analyzed by BioEdit, CLC genomic, Mega4 and DNASIS MAX software. Several polymorphisms are recognized between Naeinian goat and sheep that are presented on motif sites. In this research, the interested location, including exon I and a part of 5′, was analyzed, and genetic element comparisons were done between Naeinian goat and sheep. The number and location of probable binding sites can have a crucial role as a result of antagonistic and synergistic effects on gene regulation activities.

Introduction

Among the calcium-sensitive caseins, the αs1 fraction is undoubtedly the absolute most extensively investigated in livestock. The goat caseins (αs1-, β-, αs2 - and κ-casein) are coded by the single autosomal genes,

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CSN1S1, CSN2, CSN1S2 and CSN3, which cluster in a DNA segment of approximately 250 kbp, mapped to chromosome 6 (Rijnkels, 2002). All casein fractions are classified by high variability (Sacchi et al., 2005). So far, at least 17 alleles (A, B1, B2, B3, B4, C, D, E, F, G, H, I, L, M, N, 01 and 02) have already been identified, which are related to various levels of CSN1S1 expression in the milk. The CSN1S1 variants are sorted into 4 groups due to the basis of the milk casein alpha-S1 content: strong alleles (A, B1, B2, B3, B4, C, H, L and M) producing almost 3.5 g/l of casein alpha-S1/each; intermediate alleles (E and I; 1.1 g/l); weak alleles (D, F and G; 0.45 g/l); and null alleles (01, 02, and N) producing no αS1-casein (Bevilacqua et al., 2002; Cosenza et al., 2008; Grosclaude, 1999; Leroux et al., 1990; Ramunno et al., 2004, 2005; Rando et al., 2000) (Grosclaude, 1999) (Leroux et al., 1992; Mahé and Grosclaude, 1989; Pérez et al., 1994). A recent study showed that the high frequency of the strong genotypes is associated with the production of high fat and protein content milk and with optimal technological properties (Mastrangelo et al., 2013). CSN1S1 has been characterized by 19 exons ranging in size from 24 (exons 5, 6, 7, 8, 10, 13 and 16) to 382/388 bp (exon 19) expanded over 17.5 kb (Pérez et al., 1994). The understanding of the translational process of alpha-S1 casein gene mutants will provide new opportunities to select the best dairy livestock for the preferred milk protein producing genotype. It has been suggested that a mutation, occurring at position of −1319 of the promoter region, creates an extra putative activator protein (AP-1) binding motif in the sequence of the F allele, which can be responsible for the different expression of F and N alleles (Ramunno et al., 2005). Therefore, it is possible that using these methods, more quick and accurate selection of animal types independently of age, sex and lactation could be done in order to the different expression levels of the single alleles; thus, animals producing milk with specific chemical–physical and technological characteristics could be chosen. According to Ramunno et al. (2005), comparative analysis of the first 200 bp of the CSN1S1 promoter regions of different species showed a homology between goat and other ruminants (similarities of about 96% with cattle, sheep and yak) stronger than that observed with non-ruminants (similarities of about 88% with rabbit, 80.5% with human and 77% with rat). Common expression features suggest the existence of common motifs and a set of transcription factor binding sites in promoter regions of these genes. Multiply alignment of three to eight milk protein gene promoters showed the existence of these common motifs (Lee et al., 1987) (Laird et al., 1988); however, the question is which of those motifs are specific for the whole group of milk protein gene promoters. To date, eight transcription factors important for milk protein gene expressions and the locations of their binding sites in some of these gene promoters have been described as (1) mammary gland-specific nuclear factor (MGF), as a member of the signal transducers and activators of translation family (STAT5) (Wakao et al., 1995), which also appears to be identical to the milk protein-binding factor (MPBF) (Burdon et al., 1994); (2) mammary cell-activating factor (MAF), a member of Ets-related proteins (Welte et al., 1994a); (3) pregnancy-specific mammary nuclear factor (PMF) (Lee and Oka, 1992); (4) CCAAT: enhancer binding protein (C: EBP) (Raught et al., 1995); (5) CTF:NF1 (Li and Rosen, 1994); (6) single-stranded DNA-binding transcriptional regulator (STR) (Altiok and Groner, 1994); (7) yinyang (YY1) (Meier and Groner, 1994); and (8) glucocorticoid receptor (GR) (Welte et al., 1994b). Transcription factors are recognized sequences mostly with 6–16 bp long (Wingender, 1993). In fact, progesterone receptor (PR), activating protein-1 (AP-1), CCAAT/enhancer-binding protein (C/EBP), signal transducer and activator of transcription 5 (STAT5, originally identified as milk protein binding factors, MPBF) and pregnancy-specific mammary nuclear factor (PMF) are involved in transcriptional activation, whereas yin–yang (YY1) is involved in gene repression activity. Thus, the occurrence of various consensuses in gene promoters can indicate which transcription factors may be involved in a gene regulation activity. Previous computer analysis of milk protein gene promoters has shown their more complex structure (Malewski and Zwierzchowski, 1995). Promoter analysis has been shown that gene expression could be affected by transcriptional factors and the study about transcriptional factor binding element improves our knowledge in gene expression. This study was for characterizing of gene structure in upstream site of 5′-flanking region and exon 1 of αs1-casein gene in Naeinian goat and sheep.

Materials and methods

Animals and DNA extraction

Blood samples were randomly collected from 300 Naeinian sheep ($n = 150$) and goat ($n = 150$). Genomic DNA was extracted from blood samples using modified salting out method. Quality and quantity of extracting DNA were measured by agarose gel electrophoresis.