

Characterization of the ovine α 1-casein (CSN1S1) gene and association studies with milk protein content in Manchega sheep breed

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There are evidences showing that polymorphism of the α 1-casein (CSN1S1) gene have been associated with an effect on milk protein, casein and fat content as well as on cheese yield in ruminants. This work focuses on the characterization and evaluation of the ovine α 1-casein (CSN1S1) as a candidate gene related to protein content. Primers were designed from ovine cDNA, bovine sequences and the Sheep Genome Assembly vs 1.0. Genomic DNA from animals with extreme values for protein content (n=8, Manchega and Assaf sheep breeds) was used to search polymorphisms. BLAST software was used to confirm gene identities. Subsequently, we used ClustalW software to align and identify polymorphisms. Studies of putative regulatory elements within the promoter and potential target sites for miRNA within the 3' UTR regions were performed using TF Search and microinspector soft wares. The CSN1S1 complete genomic DNA sequence was determined (18,427-bp), including promoter and UTRs regions. Exons were identified by comparison with ovine and bovine sequences. Sequencing studies revealed 61 polymorphisms: 5 polyT, 1 poly A, 1 GT microsatellite, 2 indels and 52 SNPs. Two polymorphisms detected in the 5' flanking region were located within possible trans-acting factor binding sites, modifying a putative CdxA and GATA-1 consensus sites. The 2 SNPs located in coding regions were synonymous substitutions (exon 14 and 17). Finally, a SNP located in exon18 modify a putative target site for a miRNA (bta-miR-631). This SNP was genotyped using RFLP-PCR. No associations were found between polymorphism in exon 18 and EBVs for protein content in a daughter design comprising 13 sire families in Manchega sheep breed. Further studies are being carried out in order to test the effect of CSN1S1 promoter polymorphisms in protein content.