P3010 Characterization of 3' untranslated region (3' UTR) of *CAST* gene and its putative functional effect in beef tenderness.

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The system calpain-calpastatin (*CAPN1*-*CAST*) regulates post-mortem proteolysis and affects beef tenderness. Some SNPs in *CAST* gene have been associated with meat tenderness, including a SNP in 3'UTR region described by Barendse et al. (2002) (BTA7:g.98579663A>G on bovine genome assembly UMD 3.0). However the functional effect of this polymorphism has not been studied. The aim of this study was to search for polymorphisms with functional consequences in the 3' UTR region of *CAST* gene. Genomic DNA of the 749 bp fragment of the 3' UTR region from 49 animals with extreme tenderness estimated values and alternative genotypes for g.98579663A>G of two Spanish beef breeds (Parda de Montaña and Pirenaica) was amplified by polymerase chain reaction (PCR) for direct cycle sequencing. Eight polymorphisms were found in this region in both breeds. The majority of polymorphisms occurred as multiSNP combinations for individual subjects. Haplotype analysis identified 3 main haplotypes. *In silico*analysis using Microinspector software showed that six of them modify putative target sites of three bovine miRNA (2 for bta-miR-542-5p and 1 for bta-miR-488). The SNP g.98579663A>G modified one putative target site for bta-miR-542-5p. The multiple potential binding sites of miRNAs in large target RNAs and the energetically most favourable hybridisation site were predicted using RNAhybrid. The three main haplotypes had different minimum free energy for the different miRNAs, except for one of the bta-miR-542-5p. The sequences of the three main haplotypes were cloned into a luciferase reporter construct to evaluate the functional effect of the three haplotypes. The sequence that contains our haplotype of interest were cloned into pmirGLO (Promega) vector and confirmed by sequencing. For luciferase reporter assays, C2C12 cells, widely used as a skeletal muscle model, were transfected using JetPEI™. *Renilla* luciferase was used as a normalization control. Reporter activity was detected 48 h after transfection with the Dual-Glo Luciferase Assay System (Promega).

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ABSTRACT

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