

Session 29

Poster 21

RyR1 single nucleotide polymorphisms in *Equus caballus*

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The ryanodine receptor type 1 (RyR1) gene, located on the equine chromosome 10, encodes one of three isoforms of ryanodine receptor. It is probably associated with equine malignant hyperthermia. Rapid release of calcium (as a result of calcium channel disorders) has great influence on intracellular metabolism in endoplasmic reticulum in skeletal muscle and leads to activating metabolic processes, then hypermetabolism and even death. The mechanism of this equine disorder has not been known enough. The cause of the dysfunction is probably single missense point mutation – substitution in exon 46 of RyR1 gene. The aim of the study was to analyse RyR1 gene sequences in Anglo-Arab, Thoroughbred and Małopolski horse breed (45, 46 and 7 individuals, respectively). The fragment, which could be related with malignant hyperthermia disease, containing part of exon 45, part of exon 46 and intron 45 (according to GeneBank AH015510.2) was amplified and sequenced. Three novel polymorphic sites (SNPs) were identified in the whole population studied: A9554637G (AA frequency – 92.86%, AG – 7.%,), C9554835T (CC – 75.51%, CT – 21.43%, TT – 3.06%), C9554701T (CC – 84.69%, CT – 14.29%, TT – 1.02%). Although RyR1 exons aren't still annotated to equine chromosome 10 (GeneBank NC_009153.2), according to interspecies alignment and GeneBank AH015510.2 sequence SNPs C9554835T and C9554701T are probably located in exon sequence and SNP A9554637G is probably located in intron. Analysis on the basis of GeneBank AH015510.2 sequence also revealed, that C9554835T SNP is silent mutation. A9554637G in Thoroughbred was not identified as well as C9554701T in Małopolski horse. TT variant of C9554701T was not observed in Anglo-Arab breed. All SNPs seemed not to be breed-specific.

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Poster 22

Association and functional impact of SNPs in 3'UTR CAST gene with tenderness in cattleL.P. Iguácel¹, A. Bolado-Carrancio², G. Ripoll¹, P. Sarto¹, D. Villalba³, M. Serrano⁴, J.C. Rodríguez-Rey², M. Blanco¹, I. Casasús¹ and J.H. Calvo¹¹CITA, A. montañana, 50059, Spain, ²U. Cantabria, A. Cardenal Herrera Oría, 39011, Spain, ³U. Lleida, A. Alcalde Rovira Roure, 25198, Spain, ⁴INIA, C. la Coruña, 28040, Spain; lpiguacel@cita-aragon.es

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 the SNP BTA7:g.98579663A>G (UMD 3.1) in 3'UTR. Association results of this SNP are variable across breeds. The aim of this study was to find out the SNPs in the 3'UTR region of the CAST gene and evaluate their effect on meat tenderness in Parda de Montaña breed (n=147), as well as the functional consequences using luciferase assays. In total, 8 polymorphisms were found in this region. The majority of polymorphisms occurred as multiSNP combinations for individual subjects. Only the g.98579663A>G SNP was associated with meat tenderness at 7 days post-mortem. The AA genotype was more tender than AG genotype (P<0.05). Haplotype analysis identified 4 main haplotypes, which were not associated with meat tenderness. In silico analysis using MicroInspector software showed that 6 SNPs modify putative target sites of three bovine miRNA. The SNP g.98579663A>G modified a putative target site for bta-miR-542-5p. In order to assess the activity of the 3'UTR of CAST gene, luciferase assay within C2C12 cells was performed. A 749 bp fragment of the 3'UTR of CAST gene for each main haplotype was cloned. There were no differences between haplotypes in the activity of the luciferase, but their signal was approximately 30% lower than that of the cells transfected with empty pmirGLO vector. These findings suggest that the 3'UTR of CAST gene is an active zone. Perhaps, different miRNA binding activities among haplotypes could be found in other conditions such as other types of cell cultures, growth media or using some medium additives.

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