Analysis of a polymorphism within a TATA box of horse myostatin gene promoter in different breeds

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ABSTRACT

Myostatin or growth and differentiation factor 8 (MSTN or GDF8, respectively) is a member of the transforming growth factor-β superfamily that acts as a negative regulator of skeletal muscle development and growth. The myostatin encoding gene (*MSTN*) and the 5' regulative region in different species has been investigated. The *MSTN* gene consists of three exons and two intronic regions. In cattle, the gene has well-characterized mutations determining phenotypes with increased muscle mass. In a previous study, by alignment of nucleotide sequences of PCR products of horse *MSTN* gene, we identified some SNPs of which two T>C transitions in the promoter of the gene. The aim of this work is to study in different horse breeds the frequency of the polymorphism located 516 nt upstream of the ATG start codon. This SNP is within a TATA box sequence (NATAAAA, where N= T or C) and the comparative sequence analysis of mammals myostatin gene promoters reveals that this TATA-box motive is conserved in position and sequence among some species including cattle, sheep and goat. The point mutation is not recognised by restriction enzymes, then the genotyping was done by restriction site insertion-PCR. The primer pair was designed on the basis of the obtained nucleotide sequences. The reverse primer was modified and designed with a single nucleotide mismatched with respect to target sequence to introduces an SspI artificial restriction site when the T nucleotide occurred. The PCR products of 203 bp were digested and the resulting fragments were resolved on 3.5% agarose gel. Two hundred and six samples of DNA belonging to 18 horse breeds (Bardigiano, 20; Breton, 5; Criollo, 10; Esperia Pony, 4; Haflinger, 9; Italian Heavy Draught Horse, 24; Italian Saddle, 21; Italian Trotter, 16; Lipizzan, 11; Maremmano, 13; Murgese, 12; Norico, 10; Purebred Spanish Horse, 10; Salernitano, 12; San Fratellano, 3; Tolfetano, 7; Thoroughbred, 11 and Ventasso Horse, 8) were genotyped. The T allele occurs in all the breeds. The C allele, which eliminates TATA box motif, was detected in 11 breeds and its value frequency ranged from 0.006 (Thoroughbred) to 0.400 (Norico). The homozygous C/C genotype was detected in Haflinger, Bardigiano, Norico and Tolfetano breeds. The presence of the polymorphism within a conserved region of promoter of horse myostatin gene could affect expression gene. This marker could be used for association studies with performance traits in horses.