Expression in promoter variant of the *MEF2C* in the *Longissimus Dorsi* muscle of Polish Fresian cattle.

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Abstract—Myocyte Enhancer Factor 2 (*MEF2*) proteins are a small family of transcription factors that play pivotal role in morphogenesis and myogenesis of skeletal, cardiac, and smooth muscle cells. In vertebrates, there are four *MEF2* genes, referred to as *MEF2A*, *-B*, *-C* and *–D* that are located on different chromosomes. *MEF*2C transcripts are restricted to skeletal muscle, brain and spleen. On the basis of the sequences of the bovine chromosome 7 genomic contig, available in the GenBank database, sets of PCR primers were designed and to amplify the bovine *MEF2C* gene promoter region. Seven overlapping fragments of the bovine *MEF2C* gene were amplified and then sequenced. These fragments were composed in the 3120-bp sequence and deposited in the *GenBank* under no. GU211007. Using *MSSCP* analysis and sequencing, we investigated C/T SNP at position -1606 in the promoter region, which could be recognized with RFLP/*Bsr*I. The application of the Real-time PCR (transcript level) and Western blotting (protein level) revealed that the MEF2C gene expression level in the *longissimus dorsi* muscle LD was higher in cattle with the TT genotype than in those with the CC genotype; but the difference was not statistically significant (p=0.0712).

Index Terms-myogenesis*, MEF2C* gene, polymorphism, Real-time PCR, Western blot

I. INTRODUCTION

Skeletal muscle development involves the specification of mesodermal progenitors to a muscle fate, followed by the differentiation of those myoblasts into functional, contractile myotubes. Both of these steps are controlled by a tightly regulated transcriptional program that involves two key transcription factor families: the MyoD family of basic helix-loop-helix (bHLH) proteins and the myocyte enhancer factors 2 (*MEF2*) family are essential for both specification and differentiation of skeletal muscle. In vertebrates, there are four *MEF2* genes, referred to as *MEF2A*, *-B*, *-C* and *–D* that are located on different chromosomes [1]. Expression of the *MEF2A*, -C and -D genes marks early myogenic lineages during mouse and frog embryogenesis. *MEF2*C is the first member of the family to be expressed in the mouse with transcripts appearing in the precardiac mesoderm at day 7.5 postcoitum. In skeletal muscle muscle cells, *MEF*2C is expressed within the somite myotome beginnig at about 9.0 days postcoitus and *Mef*2A and -D are expressed immediately thereafter. After birth *MEF2*A, *MEF*2B and *MEF*2D transcripts are expressed ubiquitously, *MEF*2C transcripts are restricted to skeletal muscle, brain and spleen [2]. Despite all the available information concerning *MEF2* genes, little is

known about how this gene is regulated at the transcriptional and translational level, especially in bovine. Because of the economic importance of the bovine species to the livestock industry, it appears clearly essential to clarify some of the factors related to MEF2 genes expression.

II. MATERIALS AND METHOD

The study was performed on 136 animals (12 months) of Polish Fresian breed. Material for DNA analysis came from blood samples and was taken after slaughter and stored at -20ºC. The following PCR primers were used to amplify a 382-bp fragment of the *MEF2C* gene:F-5'actgttattgtgtactcttgtacatcc-3'; R-5'-tgacttcacttatcctgaagagttt-3'. *MEF2C* gene polymorphism was determined using MSSCP and confirmed by RFLP/*Bsr*I method. The samples for RNA extraction were obtained 15 min after slaughter from the left –side *longissimus dorsi* muscle (LD), immediately frozen in liquid nitrogen, and stored at -80ºC until analysis. RNA was isolated and the purity of RNA and the desired cDNA products of reverse transcription reaction were analyzed by 2% agarose electrophoresis and additionally with cDNA melting curve analysis. Quantification of *MEF2C* gene expression was preformed in terms of the number of *MEF2C* cDNA copies using LightCycler apparatus (Roche). The following PCR primers were used to amplify a 338 bp encompassing part of exons 2 and 3: F-5'-cgaatgcaggaatttgggaa-3'; R-5'tctgagttcgtccggctctc-3'. Expression of target genes was normalized to housekeeping gene-*SF3AI* (*splicing factor 3 subunit I*) Primes for *SF3AI* were based on Perez, Tupac-Yupanqui & Dunner (2008)- F: 5'-gcgggaggaagaagtaggag-3'; R: 5'-tcagcaagagggacacaaa-3'. For 5 dilutions of cDNA were made (1x, 4x, 16x, 64x, 256x). The efficiency of amplification was 1,982 for *SF3AI* gene and 1,833 for *MEF2C* gene. The formula of the target gene in comparison to the reference gene, in relation to mean ΔCP value of analyses group was: *ratio*= $(E_{target})^{\Delta CP}{}_{target(mean\text{-}sample)}/(E_{ref})^{\Delta CP}{}_{ref(mean\text{-}sample).}$ Western blot analysis was used to investigate MEF2C protein level in LD muscle of bulls with different *MEF2C* genotypes-CC, TT or CT. Nuclear extract proteins were resolve on an 8% SDS/polyacrylamide gel and transferred to nitrocellulose membranes. Bovine MEF2C protein was detected with rabbit anit-Human polyclonal antibody specific for MEF2C and then revealed with a goat horseradish peroxidase-conjugated secondary antibody and visualized with the chemiluminescence ECL plus Western Blotting Detection System (Amersham Biosciences). The significance of the differences in MEF2C expression level among CC, TT and CT genotypes was estimated using Duncan's test.

III. RESULTS AND DISCUSSION

The C>T transition was found by using MSSCP method and confirmed by RFLP/*Bsr*I technique in 136 Fresian bulls (Fig.1). We found 54 animals with the CC genotype, 70 with CT and 12 with TT and we used 10 animals from each genotype group (CC, TT and CT) to analyse LD muscle *MEF2C*

expression at the level of mRNA (Real-time PCR) and protein (Western Blotting). A computer analysis of the – 1614 to -1553 bp fragment of the bovine Analysis using the TESS software showed that allele C creates a SF1, GATA-1, while the G allele alternatively creates a GATA-2, GATA-3, TBP, NF-E1b binding sites, which are perfectly localized with the C/T transition at position -1606 bp and may have an influence on the expression level of the *MEF2*C gene. As shown by Real-time PCR (Fig.1) muscles of animals with genotype TT accumulated more *MEF2C* mRNA than those with the CT genotype, but the difference was not statistically significant (p=0.0761).

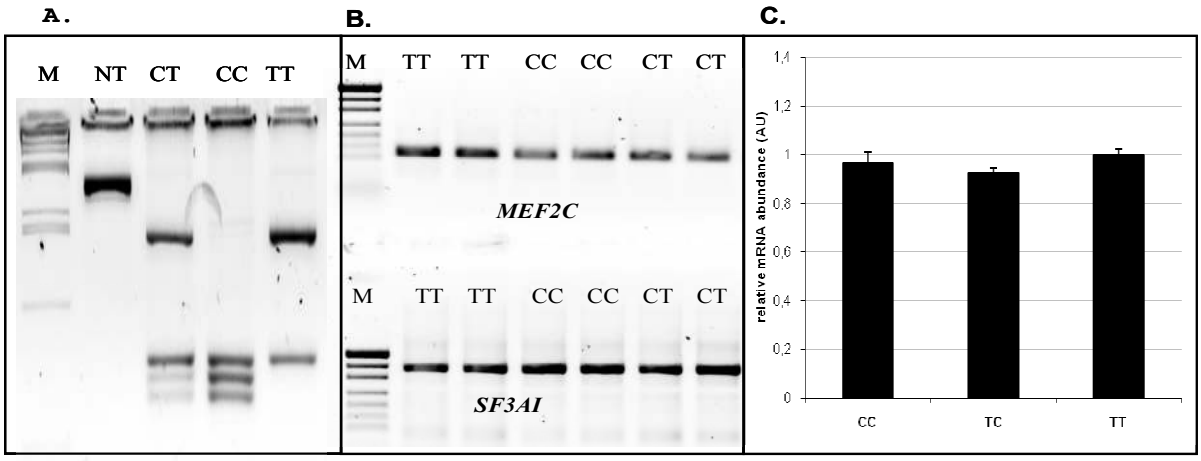

Fig1. A). RFLP genotyping of the g.-1606C>T with *Bsr*I nuclease. B).Characterization of the RT-PCR reaction done to measure expression of the *MEF2C* gen in bovine LD muscle. PCR amplification with primers specific for bovine *MEF2C* and bovine *SF3AI* genes done using bovine cDNA as template; results documented with 2% agarose gel. C). Accumulation of the *MEF2C* mRNA in LD muscle of animals with different *MEF2* genotypes (expressed in arbitary units-AU).

A further step of our study was Western blot analysis with the use of MEF2C antibodies. The results (Fig.2) showed higher expression of the *MEF2C* protein in the TT genotype muscles than in CT and CC genotype; the difference was not statistically significant. These results suggested that the studies polymorphic site could influence the expression of the bovine *MEF2C* gene.

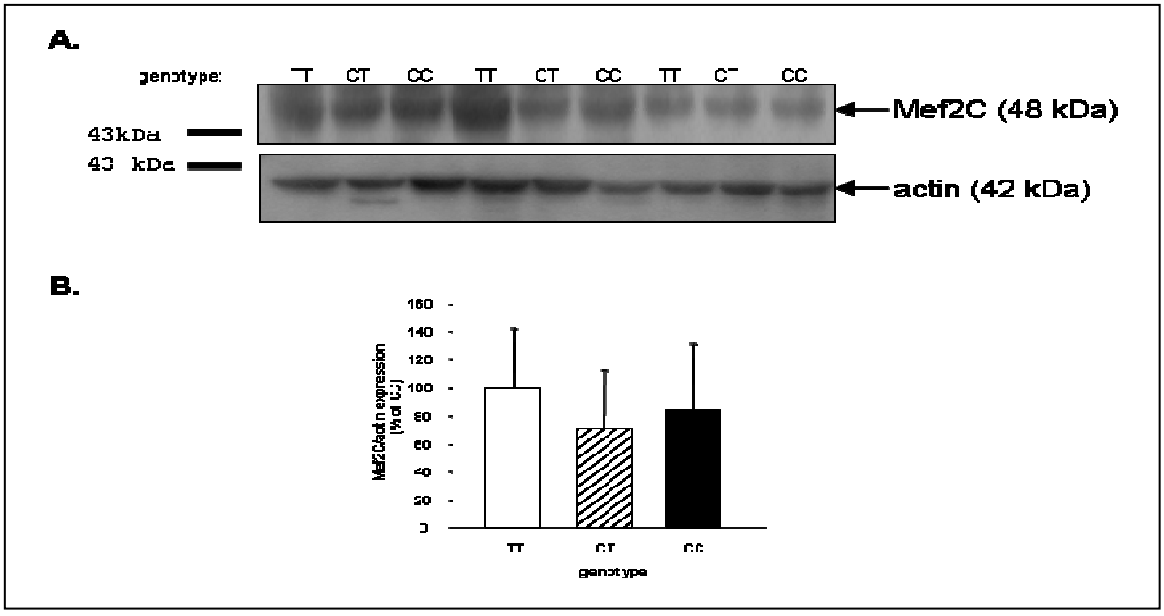

Fig 2. Western blot analysis of MEF2C protein level in LD muscle of bulls different genotypes. β-actin was used as reference. A) Blots from animals with TT, CT, CC genotype; B). The no significance of the differences in the expression level of MEF2C protein between the TT and CT, CC genotypes.

CONCLUSION

In our study we didn't find statistically significant in the relative content of *MEF2C* mRNA and expression protein between bulls of different genotypes, but we observed that the animals carrying the TT accumulated more amount of *MEF2C* mRNA and have higher expression MEF2C protein than two remaining genotypes. These results could suggest that the g.-1606C>T SNP may the effect MEF2C transcription in adult bovine muscle and that gene expression might depend on interplay between GATA-2, GATA-3, TBP, NF-E1b transcription factors, for which T allele creates binding site, respectively. However, further studied are needed to directly confirm this claim.

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