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Short communication

A new SNP in the promoter region of the porcine *MYF5* gene has no effect on its transcript level in *m. longissimus dorsi*

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Abstract. Myogenic factor 5 (myf-5) is the product of the *MYF5* gene, belonging to the *MyoD* family. This transcription factor participates in the control of myogenesis. We identified 3 new mutations in the promoter region of the gene: *A*65*C*, *C*580*T* and *C*613*T*. The aim of this study was to evaluate the influence of the *A*65*C* transversion on gene expression. The analysis was conducted on 15 Polish Large White gilts. The relative content of *MYF5* mRNA in *m. longissimus dorsi* did not differ significantly across *MYF5* genotypes (*AA*, *AC*, *CC*). This result suggests that the *A*65*C* transversion may not play an important role in the expression of the *MYF5* gene in analysed adult muscle but it abolishes a putative binding site for two transcription factors (CDP and HSF1) and creates such a site for Sp1.

Key words: gene expression, *MYF5*, pig, real-time PCR, transcription factor.

The *MYF5* gene encodes a transcription factor (TF) belonging to the MyoD family. This factor controls the processes of myogenesis and is a part of a super family of basic helix-loop-helix (bHLH) proteins, which supposedly play key regulatory roles in the development of skeletal muscles. The *MyoD* gene family consists of 4 structurally related genes: *MYOD1* (*MYF3*), *MYF5*, *MYOG* (*myogenin*) and *MYF6* (also called *MRF4* or *herculin*). The *MYF5* gene is expressed during proliferation of myoblasts (Te Pas and Visscher 1994). It comprises 3 exons: 500, 76 and 191 bp long (Te Pas et al. 1999). The structure of the regulatory region of the porcine *MYF5* gene is poorly known, but in the mouse it has been investigated and partly characterized. Chang et al. (2004) and Zammit et al. (2004) used a Yeast Artificial Chromosome (YAC) or a Bacterial Artificial Chromosome (BAC) carrying 140–190 kb of mouse genomic sequence upstream of the *MYF5* transcription start site or particular fragments of this sequence. To identify the elements involved in the regulation of the *MYF5* gene they analysed its expression in transgenic mice or transfected myoblasts (C2, C2C12) and they showed that multiple elements are required to regulate *MYF5* in the embryo and in adult skeletal muscle and that individual elements are active in different cell populations.

Three E-box sequences – a TATA-box sequence starting at nucleotides 94, 393, 477 and 811, respectively, and 2 microsatellite sequences located at positions 289 and 507 – were found in the promoter region of the porcine *MYF5* gene (nucleotide numbers according to Te Pas et al. 1999). As yet, this region was not characterized as containing putative binding sites for TFs.

The aim of the present study was to evaluate the influence of a new mutation identified by us in

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the promoter region of the *MYF5* gene on its expression.

The analysis covered 15 gilts of Polish Large White breed. The animals were maintained at a test station, National Research Institute of Animal Production, Pawłowice, all in similar environmental conditions and fed with the same commercial mixed feed. They were slaughtered at a live body weight of about 105 kg. Blood samples were collected immediately after slaughter to K_2EDTA tubes. DNA was isolated from blood leukocytes (Kawasaki 1990). Muscle samples were collected as soon as possible after slaughter and frozen in liquid nitrogen. The *MYF5* genotypes were determined as described by Urbański and Kurył (2004). RNA was isolated from 75–80 mg of muscle tissue, as demonstrated by Chomczyński and Sacchi (1987). cDNA was synthesized on a matrix RNA (2.5 μg) by using Promega reagents, according to the procedure described by the manufacturer. Glass capillaries were filled with 19 μL of a mixture (7 μL of water, 1 μL of each starter, and 10 μL Light Cycler Fast Start DNA Master SybrGreen) and 1 μL of cDNA (containing 30 ng of reverse transcribed total RNA) and the reaction real-time PCR was made on the apparatus LightCycler (ROCHE). β-actin was used as an internal control in each reaction and the mRNA expression level of the *MYF5* gene was presented as units relative to the expression of the β-actin gene. A pair of primers was designed basing upon the porcine *MYF5* gene sequence (GenBank, accession number Y17154), using Primer3 program. The sequence of PCR primers for the *MYF5* gene fragment amplification in the real-time PCR reaction was as follows: forward 5'-CTGTCTGGTCCCGAAAGAAC-3'; reverse 5'-TGATCCGATCCACTATGCTG-3'; and for the β-actin gene primer sequence followed Te Pas et al. (2000): forward 5'-GGACTT CGAGCAGGAGATGG-3'; reverse 5'-GCAC CGTGTTGGCGTAGAGG-3'.

The level of *MYF5* mRNA was expressed in arbitrary units (AU). To create standards for calculating the amplification efficiency during real-time PCR, 5 dilutions of muscle cDNA (1×, 5×, 25×, 125×, 625×) were primed separately (in different PCR tubes) for *MYF5* and β-actin as a reference gene.

We used 5 animals of each genotype and real-time PCR was repeated 3 times for each gilt tested. The hypothesis of no difference in *MYF5* expression level across genotypes *AA*, *AC* and *CC* was verified with the Duncan test.

We found 3 new mutations in the promoter region of the *MYF5* gene as compared to its sequence reported by Te Pas et al. (1999): *A*65*C* transversion, and two transitions $C \rightarrow T$ at positions: 580 and 613, respectively (Urbański and Kurył 2004). Numbering of nucleotides followed Te Pas et al. (1999). We evaluated only the influence of mutation *A*65*C* on the expression of the *MYF5* gene because for this SNP all 3 genotypes (*AA*, *AC*, *CC*) were represented. For transitions *C*580*T* and *C*613*T*, genotype TT was not observed and CT animals were rare. A similar distribution of these genotypes was also observed in Polish Landrace and synthetic line L990 pigs (Urbański and Kurył 2004).

The relative content of *MYF5* mRNA in meat samples from animals of different *MYF5* genotypes (*AA*, *AC*, *CC*) did not differ significantly. It must be mentioned that the results were very repeatable between animals of the same *MYF5* genotype (highest SD value 13.8%) and within animals (highest SD value 9.7%). These results suggest that the *A*65*C* polymorphism in the promoter of the *MYF5* gene may not affect its expression at the transcript level in an adult muscle.

A computer analysis (using the TESS program) of the fragment of the porcine *MYF5* gene – with and without mutation *A*65*C* – showed the presence of several putative TF-binding sites for each of the *MYF5* gene variants and corresponding coefficients of binding strength (L_a) are shown in Table 1. We found binding sites for two TFs in a sequence shown by Te Pas et al. (1999): CDP and HSF1, and one for Sp1 in the sequence comprising mutation *A*65*C*. Sp1 has been implicated in the activation of a very large number of genes and is thought to be involved in cellular processes, such as cell cycle regulation, chromatin remodelling and the propagation of methylation-free islands (MacLeod et al. 1994).

Table 1. Transcription factors binding to the porcine *MYF5* promoter sequence deposited in GenBank (Y17154) and comprising the *A*65*C* transversion

Sequence	Transcription factor	Binding strength
ccg**A**ccg	CDP	1.03
	HSF1	1.10
ccg**C**ccg	Sp1	1.49

Te Pas et al. (2000) studied expression of the *MyoD* family genes in postnatal muscle tissue in relation to porcine selection for growth rate and leanness. Boars from a line selected for fast

growth (F-line) or against backfat thickness (L-line) were slaughtered at the body weight of about 110 kg and biopsies were taken from 12 muscles. The F-line showed higher mRNA levels of the *MYOD1, MYF5* and *MYOG* genes than the L-line only in *m. semitendinosus*-white. Moreover, within-line evaluation revealed a systematic muscle effect for the expression level of *MyoD* genes. We did not observe any significant influence of the *A*65*C* transition in the promoter region of the *MYF5* gene on its expression in *m. longissimus dorsi*, but this transversion created a binding site for Sp1, known as a very important TF for several cell processes. Zammit et al. (2004) concluded that multiple enhancers are required to regulate *MYF5* in adult skeletal muscles, and that initiation of *MYF5* expression can be driven by elements genetically separate from those required for maintenance or later induction of expression in the same anatomical structures. Moreover, those authors suggested that different circuits may operate at adult and embryonic stages.

Recently Dutch scientists have undertaken studies on the prenatal expression pattern of genes in muscles, including *MyoD* family genes, by a microarray technique. First analyses indicated that a number of genes in several pathways – including myogenesis, energy metabolism and structural muscle genes – show differences in expression between Duroc and Pietrain embryos/fetuses (pig breeds extreme for meat quality) at several different prenatal ages (Te Pas 2004).

A similar study performed in the postnatal period on pigs of different genotypes, regarding polymorphisms in regulatory sequences of genes, could help to establish both the gene expression pattern during muscle growth and to determine the level of influence of gene variability on its expression level. A further study on the influence of *C*580*T* and *C*613*T* transitions in the promoter of the porcine *MYF5* gene on its expression should be performed on a pig breed comprising all possible genotypes (*CC*, *CT*, *TT*), which were not present within the experimental material available for the presented study.

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