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Effects of polymorphism in the bovine *PTPRQ* gene on the expression of *MYF6* and *MYF5* genes in skeletal muscle and on meat production traits in beef bulls

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ABSTRACT: The aim of the study was to characterize nucleotide sequence polymorphisms in the bovine *PTPRQ* gene, to search for their possible effect on the expression of myogenic factor 6 (*MYF6*; *MRF4*) and myogenic factor 5 (*MYF5*) genes in skeletal muscle and on meat production traits. Three novel SNPs were found in intron 35 of the bovine *PTPRQ* gene: g.200,451A>G, g.200,467T>C, g.200,480C>T (GenBank Acc. No. NW_001494990.2; counted from translation initiation site). These SNPs are placed very closely to each other (within 29 base pairs). The results showed that genotype influenced the expression of *MYF6* and *MYF5* genes in *longissimus dorsi* muscle of Limousine bulls both at the transcript and protein levels. Moreover, an association was found between the *PTPRQ* genotype and carcass traits in Limousine bulls. These findings suggest that bovine *PTPRQ* gene may contain regulatory sequences for *MRF* genes located 24 kb downstream. The results also showed that nucleotide sequence polymorphisms in the *PTPRQ* gene may influence meat production traits in beef cattle, possibly through the regulation of the *MRF* genes expression.

Keywords: receptor-type III PTP with phosphatidylinositol phosphatase activity; genotype; muscle regulatory factors; bovine; meat traits

INTRODUCTION

In vertebrates, the development of skeletal muscles depends on the basic helix-loop-helix (bHLH) myogenic regulatory factors (MRFs) encoded by the family of four conserved genes: *MYOD1*, *MYOG*, *MYF5,* and *MYF6* (*MRF4*). Experiments carried out with knock-out mice showed that MRFs take part in the regulation of muscle mass, muscle force, and cross-section area (Macpherson et al. 2011). In farm animals these factors were shown to regulate the development of musculature and to influence meat production capacity. Due to their functions, *MRF*-encoding genes are considered candidate markers for meat production in farm animals. Several studies have shown associations between sequence nucleotide polymorphisms in *MRF* genes and muscle mass and carcass traits in pigs (Liu et al. 2008), cattle (Li et al. 2004; Bhuiyan et al. 2009; Robakowska-Hyzorek et al. 2010), and chicken (Yin et al. 2011).

Muscle development needs a well-coordinated time- and place-dependent expression of *MRF*

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genes (Sabourin and Rudnicki 2000; Berkes and Tapscott 2005). Expression of each *MRF* gene is individually controlled by regulatory elements located either directly before or far upstream of the gene transcription start site.

MYF6 and *MYF5* are linked in all vertebrates (Maak et al. 2006), and the *MYF6–MYF5* locus is coordinately controlled by multiple regulatory elements. In the last two decades the linked regulation of *Myf6* and *Myf5* has been studied in transgenic mice. The studies showed that expression of these genes is driven by a combination of promoters and a number of enhancers dispersed throughout 140 kb upstream of the *Myf5* gene, buried in intronic sequences and adjacent genes (Teboul et al. 2002; Chang et al. 2004; Carvajal and Rigby 2010). Most sequences, which control the spatiotemporal expression of *MYF5*/*Myf5* and *MYF6*/*Myf6*, were located in introns of the *PTPRQ*/*Ptprq* gene (Carvajal et al. 2001; Ribas et al. 2011; Chandra et al. 2015). Maak et al. (2006) made a comparative analysis of *MYF5–MYF6* regulatory elements in different vertebrate species with enhancers previously identified in mice. They found several elements conserved between mice, human, cattle, pig, and dog, located within *PTPRQ* gene introns, up to 140 kb upstream of the *MYF5* gene and named enhancers: E1, E2, E3, E4, H1, H2, A17, and M6EH. In mouse, the H1 enhancer is located within the -58/-48 kb region important for *Myf5* expression in limb progenitors and somites. In the H1 enhancer a 145-bp regulatory element is strongly conserved among species (Giordani et al. 2007). Similarly, murine ortholog of the 70-bp enhancer element of human *PTPRQ*/*Ptprq* is required for *MYF5*/*Myf5* expression in satellite cells in adult limb muscle (Soleimani et al. 2012; Chandra et al. 2015).

Additionally, *Myf5* and *Myf6* are regulated by transcription balancing sequence (TRABS) regulating the equilibrium between enhancers and promoters of *Myf5* and *Myf6* in order to establish the regulation specificity (Carvajal et al. 2008).

PTPRQ is classified as a receptor-type III PTP with phosphatidylinositol phosphatase (PIPase) activity. The *PTPR*Q gene is not expressed in skeletal muscles and its expression is restricted to a limited number of cell types. It is expressed mostly in mesenchymal stem cells (MSCs) and its possible role is regulation of adipogenesis (Jung et al. 2009). Expression of a *PTPRQ*-like transcript was observed in cultures of bovine vascular smooth muscle cells (Borges et al. 1996). There is evidence that protein tyrosine phosphatases are involved in the regulation of skeletal-muscle myogenesis (Suryawan and Davis 2003; Lee et al. 2014). However, neither function nor expression of the *PTPRQ* gene was studied in cattle in detail.

Bovine *MYF6* and *MYF5* genes have been mapped to chromosome 5 (BTA5) q13; they are separated by 7.5 kb. The *PTPRQ* gene is also located to BTA5, approximately 24 kb 5' from *MYF6* and 33 kb from *MYF5* transcription start sites, respectively (GenBank Map Viewer database). The bovine *PTPRQ* gene extends for 231 400 base pairs (bp) and comprises 45 exons.

High similarity of *MYF5–MYF6* regulatory sequences between different species indicates that these regions have been conserved during evolution and their mutations should be rare. In the bovine *PTPRQ* gene 517 SNPs were found upon re-sequencing of the whole bovine (*Bos taurus*) genome by the BCM-HGSC consortium (GenBank SNP database). However, none of these SNPs were studied with respect to their possible phenotypic effects. Therefore, detection of functional polymorphisms in the *PTPRQ* gene and search for their possible effects on animal's phenotype could be helpful in identifying novel molecular markers for meat production traits in cattle.

The objective of our study was to find out SNPs in the bovine *PTPRQ* gene fragment containing putative H1 enhancer element and to test for their possible effects on the expression of *MYF6* and *MYF5* genes and for their association with meat production traits in cattle.

MATERIAL AND METHODS

Animals and DNA samples. To investigate the genotype and allelic frequencies, blood samples were collected from 190 unrelated bulls of different breeds: Hereford (HER, n = 31), Limousine (LIM, n = 31), Polish Holstein-Friesian (HF, n = 82), and Polish Red (PR, n = 46). Genomic DNA was subsequently extracted from blood samples by the method of Kanai et al. (1994) and stored at –20°C. All procedures carried out on animals were approved by the Local Ethics Commission, permission Nos. 3/2005 and 85/2006.

Longissimus dorsi (LD) muscle samples (n = 190) were obtained from 12-month-old bulls at local

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abattoir, immediately after slaughter. Fragments derived from different parts of the muscle were flash frozen in liquid nitrogen and then stored at 80°C until use.

A group of 62 beef bulls only (LIM, n = 31 and HER, n = 31) was used for association analysis of the *PTPRQ* gene polymorphisms with meat traits. They were fed *ad libitum* silage, hay, and concentrate, with constant access to water. After 24-hour fasting, the bulls were slaughtered. The carcasses were chilled for 24 h at 4°C. Valuable cuts (round, shoulder, tenderloin, best ribs + fore ribs) were obtained from the right halves of carcasses and dissected into lean, fat, and bone (Oprzadek et al. 2001).

The genotype–trait association of the data on carcass composition was analyzed by the General Linear Model procedure of SAS (Statistical Analysis System, Version 9.4, 2013) as follows:

$$y_{ijkl} = \mu + G_i + B_j + YS_k + \beta(x_{ijkl} - x) + e_{ijkl}$$

where:

Y_{ijkl} = mean value of the trait
μ = general mean
G_i = fixed effect of *PTPRQ* genotype (i = 1, 2 or combined genotype i = 1, ..., 4)
B_j = fixed effect of breed (j = 1, 2)
YS_k = fixed effect of year and season (k = 1, ..., 10)
$\beta(x_{ijkl} - x)$ = regression on body weight at slaughter
e_{ijkl} = random error

Treatment means were generated using the LSMeans and PDIFF options and separated using Bonfferoni's test. Significance was inferred at $P \leq 0.05$.

PTPRQ gene polymorphism. The PCR primers were designed based on the bovine *PTPRQ* gene sequences from *Bos taurus* chromosome 5 genomic contig (GenBank Acc. No. NW_001494990.2), using Primer 3 program (http://frodo.wi.mit.edu/cgibin/primer3/primer3_www.cgi). Primer sequences and the length of PCR products and their gene locations are given in Table S1.

The polymerase chain reactions were performed using the following PCR mix (10 µl): 0.8 µl of 10µM forward and reverse primers, 0.25 U of HotStarTaq® DNA Polymerase (Qiagen GmbH, Hilden, Germany), 1 µl of 10X polymerase buffer, dNTPs each at concentration of 2.5mM, 1.5mM final concentration of $MgCl_2$, 1.25 µl Q-Solution, and approximately 100 ng genomic DNA. The following PCR protocol was used: initial denaturation for 15 min at 90°C, followed by 28 cycles of 1 min at 95°C, 40 s at 55°C, 50 s at 72°C, and a final elongation step – 5 min at 72°C. The PCR reactions were carried out in a MJ TETRAD thermocycler (Bio-Rad Laboratories, Hercules, USA).

After purification with the GenElute PCR Clean Up Kit (Sigma-Aldrich, St. Louis, USA), the PCR products were sequenced in an ABI 377 Sequencer (Applied Biosystems, Foster City, USA). The sequencing of 80 animals (HF, n = 9; PR, n = 9; LIM, n = 31, HER, n = 31) was done at the Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw. The sequences were analyzed using the Chromas program (www.technelysium.com.au/chromas.html).

For genotyping, the PCR products were digested for 3 h with 5 U of a respective restriction endonuclease: *Hpy*CH4IV or *Rsa*I (New England Biolabs, Ipswich, USA). The restriction products were separated by electrophoresis in 2% agarose gel (Sigma-Aldrich) with ethidium bromide in TBE buffer. Bands were visualized and documented by the Molecular Imager System FX (Bio-Rad Laboratories).

Expression of MRF genes. Total RNA was isolated with TRIzol® Plus RNA Purification Kit (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. The quantity and quality of RNA was estimated by Nanodrop (Nanodrop Technology, Wilmington, USA) and 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA). Only those samples with A_{260}/A_{280} ratio between 1.8 and 2, and RIN 8.8–10 were further analyzed. 2 µg of RNA was reverse transcribed to cDNA using M-MLV Reverse Transcriptase (Promega Corp., Madison, USA) according to the procedure given by the manufacturer.

Real-time PCR was performed using SYBR® Green PCR Master Mix technique. TATA box binding protein (*TBP*) gene was chosen for normalization of expression of *MYF6* and *MYF5* genes as the most stably expressed in bovine muscle (Robakowska et al. 2010). The real-time polymerase chain reactions were performed using the following mix: 0.8 µl of 10µM forward and reverse primers; the Power SYBR® Green PCR Master Mix (added according to the manufacturer's protocol); 500 ng cDNA used as a template. PCR primers matching exons 1 and 3 of the *MYF6* and *MYF5* genes were used (Table S1). PCR amplification was performed

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using a 7500 ABI PRISM apparatus (Applied Biosystems). PCR conditions were as follows: initial denaturation at 95°C for 10 min, 40 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 40 s. Standard curves were prepared for all pairs of primers (*TBP*, *MYF5,* and *MYF6*) and efficiency of each reaction was calculated. The PCR products were analyzed electrophoretically on 1.5% agarose gel and through melting curve analysis to check amplification specificity. The results were calculated using the mathematical formula for relative mRNA quantification in real-time PCR given by Pfaffl (2001). Expression results were statistically evaluated by Student's *t*-test, by the GraphPad Software Prism (Version 5, 2007) with *P*-value < 0.05 and *P* < 0.01 being considered statistically significant. Experiments were performed in triplicate for each sample, and error bars indicate the standard error of the mean (SEM).

Western blot analysis. For Western blot analysis, total proteins (100 ug) were prepared from frozen LD muscle tissue of the animals used for MYF5 and MYF6 expression analysis. Extracted proteins were diluted (1 : 4, v.v.) in Laemmli buffer (100mM Tris-HCl, pH 6.8, 4% sodium dodecyl sufate, 20% glycerol, 200mM dithiothreitol, bromophenol blue) and electrophoretically resolved in 9% polyacrylamide gels. Proteins were electroblotted onto Hybond-ECL nitrocellulose membranes (GE Healthcare Life Sciences, Amersham, UK). The membranes were initially blocked by gentle agitation in Tris buffered saline (TTBS) (50mM Tris/HCl, pH 7.6, and 150mM NaCl, 0.15% Tween 20) containing 5% fat-free milk powder for 1 h at room temperature followed by overnight incubation at 4°C with primary antibodies sc-301 or sc-302 – purified rabbit antibodies raised against C-terminal peptides of human MYF6 and MYF5, respectively (Santa Cruz Biotechnology, Dallas, USA). Membranes were then washed in TTBS and incubated with peroxidase-conjugated anti-rabbit secondary antibodies (Santa Cruz Biotechnology) for 1 h at room temperature. Immunoreactive bands were detected using the ECL Plus Western blotting detection system (GE Healthcare Life Sciences). Quantification of MYF5 and MYF6 was performed relative to bovine TUBULIN and ACTIN respectively. Blots were visualized and quantified using densitometry with a Molecular Imager with Quantity One analysis software (Bio-Rad Laboratories).

Analysis of DNA sequence in MYF6, MYF5, and PTPRQ loci. The sequence of bovine loci encoding PTPRQ, MYF5, and MYF6 was determined by comparison of *Bos taurus* clone working draft sequence AC132076.3 (Maak et al. 2006) with the nucleotide contig directly retrieved from GenBank: NW_001494990.2 using BLAST algorithm. Further comparisons (alignments) were done between bovine and murine *Ptprq* sequences to find putative regulatory motifs for *MYF5* and *MYF6* genes, also using BLAST algorithm.

The sequence of the bovine *PTPRQ* gene was analyzed for the presence of putative transcription factor binding motifs, using CONSITE (http://asp.ii.uib.no: 8090/cgi-bin/CONSITE/consite/), JASPAR software (http://jaspar.genereg.net/), and TESS (http://www.cbil.upenn.edu/cgi-bin/tess/tess) databases.

RESULTS

Identification of genetic variants of PTPRQ gene. Experiments were performed to search for nucleotide sequence polymorphisms in the bovine *PTPRQ* gene intron 35, exon 36, and intron 36, where the putative H1 enhancer element and 145 kb conservative sequence are located. A total of 1,356 bp sequence of bovine *PTPRQ* gene was resequenced, thus resulting in the detection of three novel variants located in intron 35: g.200,451A>G (RFLP-*Hpy*CH4IV), g.200,467T>C (RFLP-*Rsa*I), and g.200,480C>T (Figure S1). Two of these polymorphisms could be genotyped by restriction fragment length polymorphism (RFLP), the third one is placed in a cutting site for a restriction enzyme – *Tsa*I, however RFLP was not possible because of the same recognition sequences in close vicinity. The SNPs are closely located to each other, within 29 base pairs, and thus are possibly linked. These polymorphisms are located about 58 kb 5' of the *MYF6* gene and about 67 kb 5' of the *MYF5* gene transcription start sites, respectively.

One hundred and ninety bulls and cows of four breeds – Hereford (HER, *n* = 31), Limousine (LIM, *n* = 31), Polish Holstein-Friesian (HF, *n* = 82), and Polish Red (PR, *n* = 46) were genotyped by RFLP (g.200,451A>G and g.200,467T>C). For g.200,480C>T genotype 31 LIM and 31 HER bulls as well as samples of 9 animals of HF and PR breeds were genotyped with DNA sequencing. The *ATC* allele and *AA-TT-CC* genotype prevailed in all breeds

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tested; 19 LIM bulls, 21 HER bulls, all genotyped HF and 44 PR animals were *AA-TT-CC* homozygotes, 22 were *AG-TC-CT* heterozygotes (PR, *n* = 2; LIM, *n* = 10; HER, *n* = 10). The *GG-CC-TT* homozygous genotype was very rare appearing only in two LIM bulls. Based on the two polymorphic sites genotyped with PCR-RFLP and the sequenced sample of HF animals, there is a high probability that HF cattle are monomorphic (*AA-TT-CC* homozygotes) with respect to the *PTPRQ* genotypes. In the tested group of PR cattle two *AG-TC-CT* heterozygotes were found. The genetic analysis confirmed that the three closely placed SNPs form a closely linked combined genotype.

Putative transcription factor binding sites in the bovine PTPRQ gene. Putative transcription factor binding sites (TFBS) were searched in the bovine *PTPRQ* gene fragments containing the three SNPs. Three TFBS were found co-located or adjacent to the SNPs under study (Figure S1; Table 1). A putative androgen receptor binding site was created by substitutions T→C and C→T at positions 200,467 and 200,480, respectively. For each mutation separately different TFBS were identified. The g.200,467T>C transition generates putative binding site for C/EBP homologous protein (CHOP), which is an endoplasmic reticulum stress-inducible protein. The g.200,480C>T transition creates a putative binding site for hepatocyte nuclear factor 3 (HNF-3).

Effect of PTPRQ polymorphism on the expression of the MYF6 and MYF5 genes. The expression (at transcript and protein level) of the *MYF5* and *MYF6* genes was measured in LD muscle of bulls with different *PTPRQ* genotypes at the three SNPs. Muscle samples of 19 LIM bulls of the *PTPRQ* genotype *AA-TT-CC* and 10 of the genotype *AG-TC-CT*, and of 21 HER bulls of the *PTPRQ* genotype *AA-TT-CC* and 10 of the genotype *AG-TC-CT* were studied. Differences were found in *MYF6* and *MYF5* transcripts levels in the skeletal muscle between *PTPRQ* genotypes (Figure 1). The homozygous *AA-TT-CC* LIM animals showed *MYF5* mRNA significantly higher than those with the genotype *AG-TC-CT* ($P < 0.01$). No difference in *MYF5* transcript level was shown in HER bulls between *PTPRQ* genotypes.

The opposite tendency was shown for the *MYF6* gene expression; the homozygous LIM bulls with *AA-TT-CC PTPRQ* genotype had less *MYF6* mRNA in the muscle samples than those with *AG-TC-CT* (Figure 1); the difference was statistically signifi-

Table 1. Putative transcription factor binding motifs co-localizing with the SNPs found in the bovine *PTPRQ* gene

SNP	Localization	Putative transcription factor	Consensus	Created with	Recognition sequences with SNPs
g.200,467T>C g.200,480C>T	intron 35	AR	GGA/TACANNNTGTTCT (Wyce et al. 2010) RKWCANNNYGTTCT (Roy and Chatterjee 1995)	C	AAGGTA**C**/**T**AGCATGTTATAA**C**/**T**
g.200,467T>C	intron 35	CHOP-cEBP	TGCTGCAATAAG (Pietas et al. 2002)	C	TA**C**/**T**AGCATGTT
g. 200,480C>T	intron 35	HNF-3β	YYAATRDKTRAY (Jaspar, database)	T	ATAA**T**/**C**TGCTGGT

AR = androgen receptor; CHOP-cEBP = C/EBP homologous protein; HNF-3β = hepatocyte-nuclear factor 3β
symbols of nucleotides are given according to IUPAC code: N = A,C,G,T; R = G or A; K = G or T; W = A or T; Y = C or T
SNPs variants in the sequence are bolded

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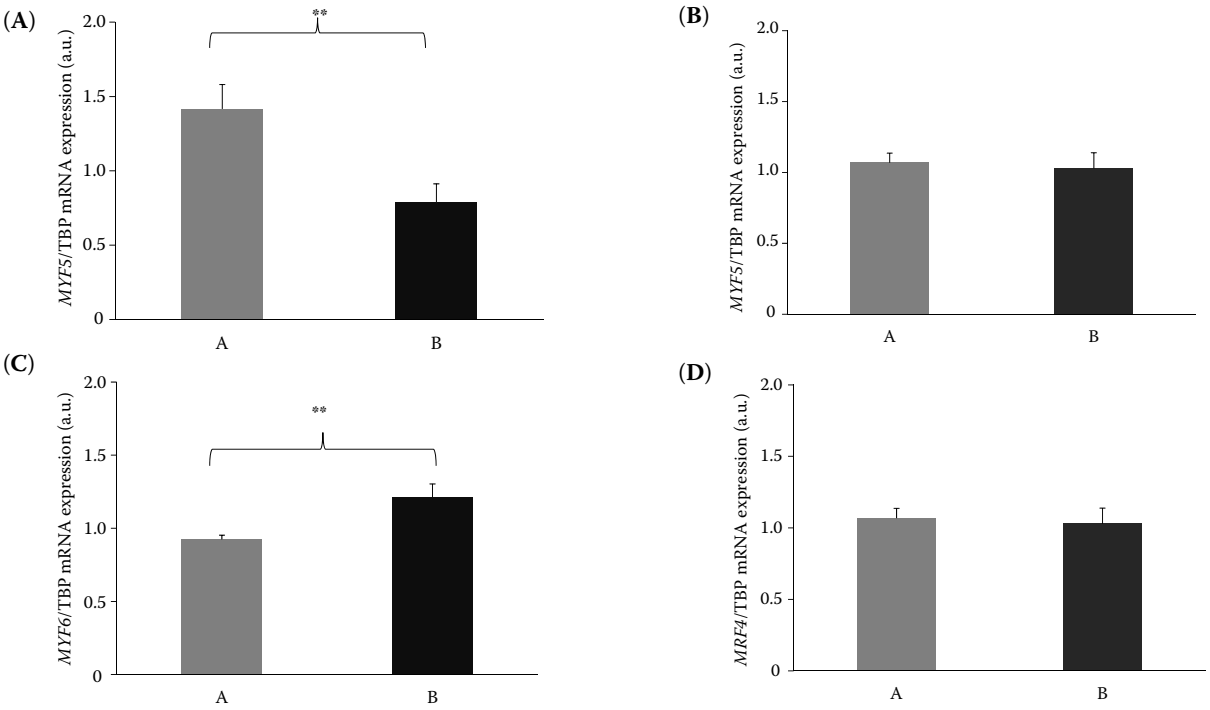

Figure 1. Relative content of *MYF5* and *MYF6* mRNA in *longissimus dorsi* muscle of Limousine and Hereford bulls differing in *PTPRQ* genotype

(**A**, **C**) Limousine bulls, *MYF5* and *MYF6* gene expression, respectively; (**B**, **D**) Hereford bulls, *MYF5* and *MYF6* gene expression, respectively

A = *AA-CC-TT* combined genotype, B = *AG-TC-CT* combined genotype

means are shown (in arbitrary units relative to *TBP* gene) ± SEM obtained from muscle samples of 19 Limousine bulls of the *PTPRQ* genotype *AA-TT-CC* and 10 of the genotype *AG-TC-CT*; from muscle samples of 21 Hereford bulls of the *PTPRQ* genotype *AA-TT-CC* and 10 of the genotype *AG-TC-CT*. Real-time PCRs were run in triplicates

$^{**}P \leq 0.01$

cant at $P < 0.01$. The transcript level of *MYF6* gene does not indicate significant differences between *PTPRQ* genotypes in Hereford bulls (Figure 1).

Protein extracts from LD muscle of LIM and HER bulls carrying *AA-TT-CC* or *AG-TC-CT PTPRQ* genotypes were used to assess MYF5 and MYF6 protein levels. For *AA-TT-CC* genotype LIM bulls the relative MYF5 protein was higher than that for *AG-TC-CT* genotype (Figure 2A, B). The MYF6 protein content in the muscle of *AG-TC-CT* genotype animals was higher comparing with the *AA-TT-CC* genotype (Figure 2C, D). No differences were found

Table 2. Effect of the *PTPRQ* genotype on carcass traits in 12-month-old Limousine and Hereford bulls

Item	Limousine		Hereford	
	AA-TT-CC (n = 19)	*AG-TC-CT* (n = 10)	*AA-TT-CC* (n = 21)	*AG-TC-CT* (n = 10)
Live body weight at slaughter (kg)	431.1 ± 4.1	426.9 ± 4.0	414.5 ± 2.4	418.2 ± 3.7
Weight of valuable cuts (kg)	82.1 ± 0.3^{A}	79.5 ± 0.5^{A}	67.3 ± 0.4	67.8 ± 0.6
Weight of meat in valuable cuts (kg)	64.0 ± 0.4^{B}	61.3 ± 0.5^{B}	45.8 ± 0.4	46.7 ± 0.7
Weight of fat in valuable cuts (kg)	6.5 ± 0.2	6.8 ± 0.4	9.4 ± 0.3	8.9 ± 0.4
Weight of bone in valuable cuts (kg)	11.7 ± 0.2	11.7 ± 0.3	12.2 ± 0.1	12.3 ± 0.2
Carcass dressing percentage (%)	60.9 ± 1.4	63.6 ± 2.1	56.3 ± 0.4	55.9 ± 0.6

values are given as Least Squares Means (phenotypic means) ± standard error

values with the same superscript within a row significantly differ ($^{A}P = 0.0002$, $^{B}P = 0.0004$)

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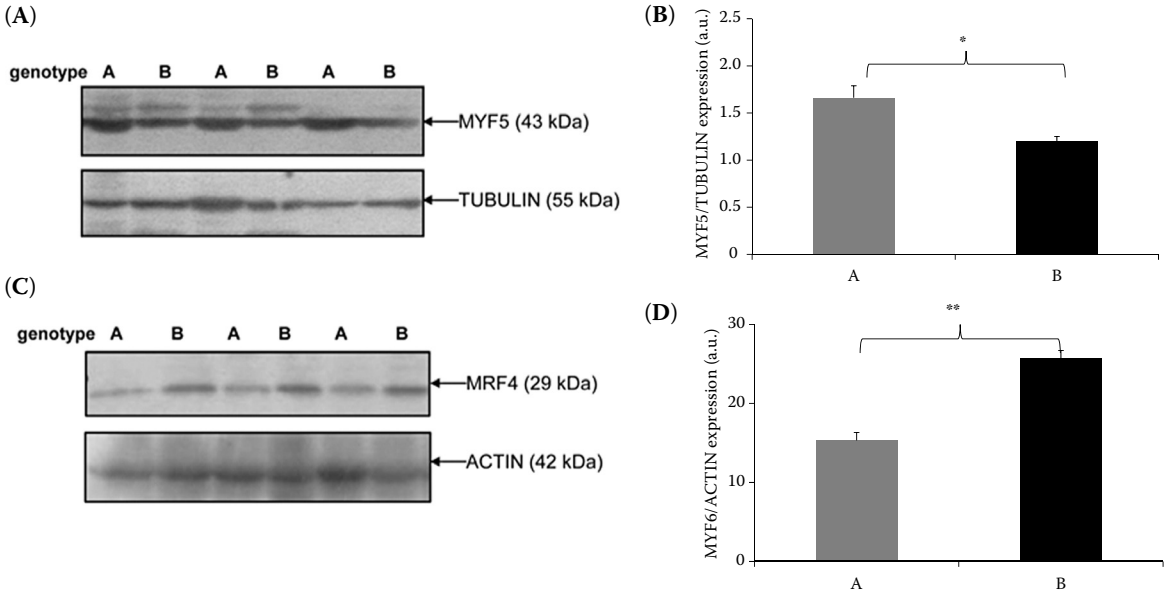

Figure. 2. Expression of MYF5 and MYF6 (MFR4) proteins in *longissimus dorsi* muscle of Limousine bulls differing in *PTPRQ* genotype

Western blotting gel showing MYF5 and TUBULIN (**A**) and MYF6 (MRF4) and ACTIN (**B**); relative MYF5 protein level (**C**) and MYF6 (MRF4) level (**D**) – densitometric analysis of data from Western blots

A = *AA-CC-TT* combined genotype, B = *AG-TC-CT* combined genotype

means are shown (in arbitrary units relative to bovine TUBULIN or ACTIN used as a reference) ± SEM obtained from the muscle samples of 3 Limousine animals of the *AA-TT-CC* genotype and 3 of the *AG-TC-CT* genotype

$^{**}P \leq 0.01$, $^{*}P \leq 0.05$

in MYF6 and MYF5 protein level between *PTPRQ* genotypes for HER bulls (Figure 3).

Effect of PTPRQ gene polymorphism on meat production traits. Thirty-one 12-month-old LIM and 31 HER bulls were used to study the associations between *PTPRQ* gene polymorphism and body weight and carcass traits (Table 2). No effect of *PTPRQ* genotypes on live body weight was shown. No significant associations were also found between *PTPRQ* genotypes and meat traits in HER bulls. However, the LIM bulls with the *AA-TT-CC* genotype had a higher weight of valuable cuts in carcasses (+ 2.6 kg vs *AG/TC/CT*; $P = 0.0002$) and weight of meat in valuable cuts (+ 2.7 kg vs *AG/TC/CT*; $P = 0.0004$).

DISCUSSION

In the present study, three SNPs were identified in the bovine *PTPRQ* gene intron 35, in which putative *cis* regulatory elements for *MYF6* and *MYF5* genes are located. The *PTPRQ* polymorphism was found mostly in beef cattle – HER and LIM. A comparative analysis of the bovine *PTPRQ* partial sequence with its mouse counterpart (Maak et al. 2006) showed that the SNPs are located closely to H1 enhancer element and close to the 145-bp sequence conserved between species, containing a regulatory element, which enhances *Myf5* transcription in mice embryonic limb and somites (Giordani et al. 2007). Moreover, they lie inside the -58/-48 kb region, which regulates spatiotemporal expression of *MYF5* and *MYF6* in dorsal and ventral myotome (Pin and Konieczny 2002).

In the present study, for the first time the effect of *PTPRQ* gene polymorphisms on meat traits was shown in cattle. Significant associations were found between combined genotypes of *PTPRQ* and carcass traits – weight of valuable cuts and weight of meat in valuable cuts in LIM bulls; no such associations were found in HER bulls. Moreover, the effect of nucleotide substitutions in the *PTPRQ* gene on the expression of MYF5 and MYF6 in the bovine skeletal muscle was shown. Interestingly, the differences were observed only in LIM bulls but not in HER, thus suggesting that a breed-dependent specificity exists in regulation of MRFs expression, which may lead to different regulation of muscle growth and phenotypic diversity. LIM and HER

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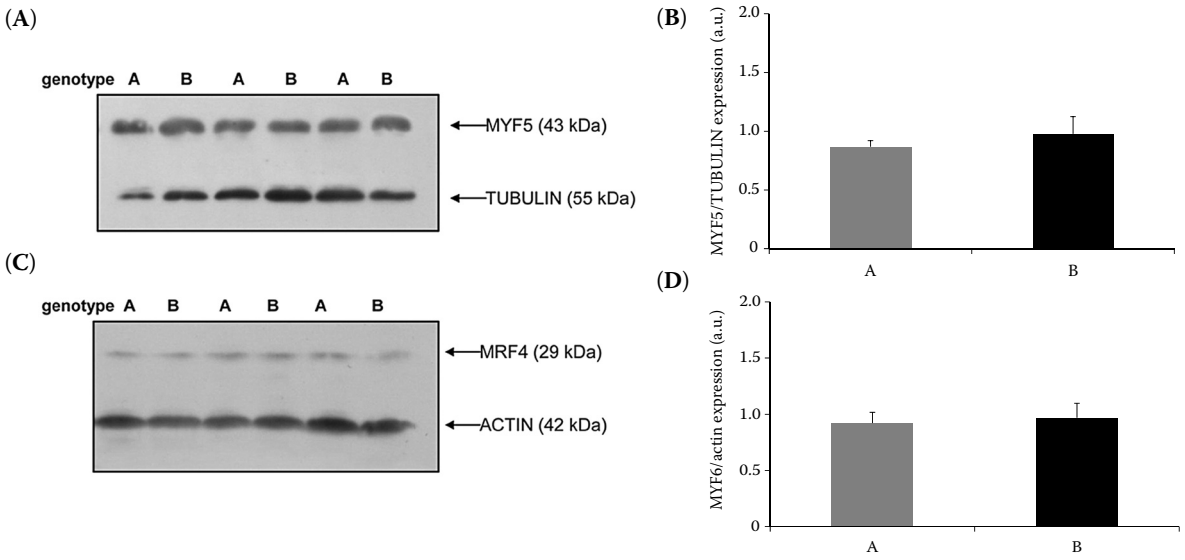

Figure 3. Expression of MYF5 and MYF6 (MRF4) proteins in *longissimus dorsi* muscle of Hereford bulls differing in *PTPRQ* genotype

Western blotting gel showing MYF5 and TUBULIN (**A**) and MYF6 (MRF4) and ACTIN (**B**); relative MYF5 protein level (**C**) and MYF6 (MRF4) level (**D**) – densitometric analysis of data from Western blots

A = *AA-CC-TT* combined genotype, B = *AG-TC-CT* combined genotype

means are shown (in arbitrary units relative to bovine TUBULIN or ACTIN used as a reference) ± SEM obtained from the muscle samples of 3 Hereford animals of the *AA-TT-CC* genotype and 3 of the *AG-TC-CT* genotype

are both beef breeds, but they differ in the time of maturity and intramuscular fat content (Sadkowski et al. 2009a). Also transcriptomic analyses confirmed different regulation of muscle growth in LIM and HER bulls, as shown by Sadkowski et al. (2009b). Only 48 genes out of 462 analyzed were similarly expressed in both beef breeds, after comparing the transcriptomes of Limousine and Hereford *semitendinosus* muscle.

There is little documentation of the role of MRFs in the later fetal and adult muscle development, opposite to well-known function of these factors in embryonic and early fetal myogenesis. In mice, the *Myf6* transcript is still highly expressed in postnatal muscle, when the transcripts of other MRFs decline, suggesting that MYF6 plays an important role in adult skeletal muscle. Because of the generally low expression level of *MYF5*, its over-expression in *AA-TT-CC* genotype LIM bulls found in the present study could be interesting in determining a possible effect on muscle fibre growth. It could be similar to the enhancing mouse *Myf5* expression in quiescent satellite cells of the adult soleus limb muscle driven by regulatory element ECR111, located in *Ptprq* gene (Soleimani et al. 2012).

Previously we showed that in 12-month-old HF bulls the g.-723G>T transition located within the promoter region of the *MYF5* gene influenced the gene expression in the LD muscle and was associated with sirloin weight and fat weight in sirloin in carcasses of HF cattle (Robakowska-Hyzorek et al. 2010).

Single nucleotide polymorphisms in regulatory regions may have variable effects on gene expression depending on their localization relative to binding sites of the transcription factors. In some cases SNPs can change phenotypic diversity by creating or destroying the binding site for important transcription factors. In the present study we analyzed nucleotide substitutions in the bovine *PTPRQ* gene located within or adjacent to the putative transcription factor binding sites (TFBS). The nucleotide substitutions either created new or diminished existing TFBS in the *PTPRQ* gene, which may possibly result in decreased or increased transcription levels of *MRF* mRNAs. We found that these SNPs are co-located with the putative binding site for androgen receptor (AR), C/EBP homologous protein (CHOP), and hepatocyte nuclear factor 3 (HNF-3). We suppose that at least some of the SNPs may be functional. Several

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reports showed that the androgen-AR signalling pathway takes part in skeletal muscle development. Farm animal studies indicated that androgens increased lean mass, strength and protein synthesis in skeletal muscles (Lee 2002), however, molecular mechanisms of these effects are still unclear. C/EBP homologous protein (CHOP) is an endoplasmic reticulum stress-inducible protein important for the regulation of programmed cell death, and thus can possibly participate in muscle remodelling during development. Several genes involved in the muscle wasting have putative C/EBP-binding sites in their regulatory sequences (Penner et al. 2002). HNF-3β is not expressed in skeletal muscle (Berger and Sanders 2000), however some studies showed that HNF-3 could bind to smooth muscle-specific gene promoters (Hoggatt et al. 2000).

Our results show that differences in MYF5 and MYF6 expression may be influenced by nucleotide substitutions (SNPs) located in the intron of the *PTPRQ* gene and also suggest that both genes are coordinately controlled by the regulatory sequences located away from their promoters and coding regions. At the moment, it is difficult to explain the molecular mechanisms of such regulation. We only confirmed in cattle that the sequences located the *PTPRQ* close to the putative H1 enhancer and the 145-bp regulatory element could influence expression of the downstream located *MRF* genes. In cattle, *MYF6*, *MYF5,* and *PTPRQ* genes are located on chromosome 5, in which quantitative trait loci (QTL) for muscle growth traits have been reported (MacNeil and Grosz 2002; Li et al. 2004). We showed that in LIM bulls the genotype of three SNPs in the *PTPRQ* gene is associated with weight of valuable cuts and weight of meat in valuable cuts, the *AA-TT-CC* homozygotes showing significantly higher results than heterozygotes. The differences were statistically highly significant ($P < 0.001$). These results suggest possible role of the *PTPRQ* polymorphism in influencing bovine muscle mass. Such polymorphisms may influence gene expression levels and thus determine differences in animals' phenotype. However, further studies must be performed in other unrelated bovine population to validate the association obtained in this study.

CONCLUSION

In this report, we have identified three novel SNPs in bovine *PTPRQ* gene found mostly in beef cattle breeds. These mutations show influence on transcript level of *MYF5* and *MYF6* genes, also on MYF5 and MYF6 proteins level measured in *longissimus dorsi* muscle in LIM bulls, but not in HER bulls. This combined genotype was also associated with meat quality traits, in particular weight of valuable cuts. This gene region is a potential target for controlling *MYF5* and *MYF6* expression. It is still not clear how regulatory elements in this locus interact with *MYF5* and *MYF6* gene promoters. Identification of nucleotide sequence polymorphisms in regulatory elements of these candidate genes may lead to better understanding the differences between animals. Such polymorphisms may influence gene expression levels and thus determine differences in animals' phenotype.

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