

An association between the C>T single nucleotide polymorphism within intron IV of osteopontin encoding gene (*SPP1*) and body weight of growing Polish Holstein-Friesian cattle*

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A relation was studied between body weight measured at the age of 3, 6 and 12 months in Polish Holstein-Friesian (HF) heifers (n=111) and young bulls (n=87) and C/T polymorphism within intron IV of bovine osteopontin encoding gene (*SPP1*). Three half-sib (HS) families were considered, each sired by heterozygous C/T sire. Significant association was found of *SPP1* C>T SNP with body weight in all the analysed HS progeny groups of young heifers and bulls. Within young bulls the differences were identified (P≤0.05) in body weight between the *SPP1* genotypes (8514C/C, 8514C/T, 8514T/T) in month 3, 6 and 12 of age. Within heifers, however, the differences (P≤0.05) were found in the progeny groups aged 6 and 12 months. Moreover, when data from bulls and heifers were pooled (n=198) the highly significant effect (P≤0.01) of *SPP1* genotype on body weight was observed at the age of 6 and 12 months.

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An effect was revealed of allele *T* as a favourable allele related to higher body weight in both young bulls and heifers. The presented results suggest that *SPP1 C>T* SNP marker might be considered as candidate gene for intensive growth in Polish HF cattle and could further be investigated in large co-segregating HS families or other experimental designs.

KEY WORDS: body weight gain / cattle / growth / nucleotide polymorphism / osteopontin

Osteopontin (OPN) was first described as a secreted, 60-kDa transformation-specific phosphoprotein [Senger *et al.* 1979] mediating cell matrix interactions and cellular signaling through binding with integrin and CD44 receptors. The protein in question is expressed in numerous tissues [Denhardt and Guo 1993]. Accordingly, secreted phosphoprotein 1 (SPP1) was introduced as an alternative term to reflect the broader functional role of the factor. Bovine *SPP1* gene comprises 7 exons spanning about 7 kb of genomic DNA (GenBank accession number NW 255516). In a newly assembled bovine whole-genome sequence database (<http://pre.ensembl.org/Bostaurus/index.html> based on Btau 3.1) the bovine *SPP1* (NC 007304.2) gene is composed of 6961 bp in total length: 1331 bp in processed length and 278 in protein product length (Fig. 1 – ‘A’). In relation to milk and beef production traits, recent studies by Schnabel *et al.* [2005], Leonard *et al.* [2005], Allan *et al.* [2007], Khatib *et al.* [2007], and White *et al.* [2007] led to identification of several mutations mainly in intronic region of the bovine *SPP1* encoding gene (Fig. 1 – ‘B’).

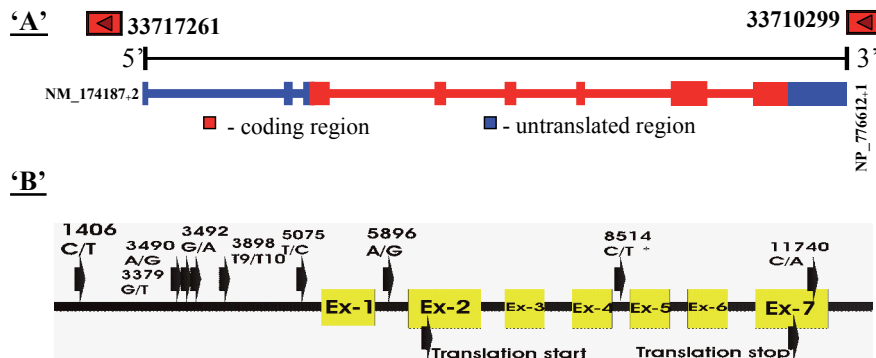


Fig. 1. ‘A’ represents the organization of *SPP1* gene of cattle in newly assembled bovine genome sequence database based on Btau 3.1 (http://pre.ensembl.org/Bos_taurus/index.html). ‘B’ shows the identified SNP within bovine *SPP1* gene.

In dairy cattle *SPP1* gene was identified as a positional candidate gene for both milk and beef production traits, harbouring the QTL region (at the position of 55.4 cM, where BM143 microsatellite marker is mapped) on bovine chromosome 6 (BTA-6) – Schnabel *et al.* [2005], Khatib *et al.* [2007]. The former group of authors proposed

that a SNP upstream of bovine *SPP1* gene was a positional candidate polymorphism explaining a QTL on BTA-6 that affected milk traits. In the following years White *et al.* [2007] were able to show significant effects of *SPP1* polymorphism in two US bovine populations on post-weaning growth. However, birth weight and weaning weight showed no significant associations with *SPP1* polymorphism. The lack of association with growth trait was somewhat puzzling given the known biology of *SPP1* gene in terms of tissue growth [Rangaswami *et al.* 2006] and embryonic growth [Weintraub *et al.* 2004]. Recently, Allan *et al.* [2007] have extended the White's *et al.* [2007] work by further evaluating the association of *SPP1* gene polymorphism with body growth rate in a large cattle population spanning multiple generations. They found that the associations of the *C>T* SNP *SPP1* marker with birth weight ($P \leq 0.006$), weaning weight ($P \leq 0.007$) and yearling weight ($P \leq 0.003$) were in accordance with the earlier reported effects of *SPP1* genotype on yearling weight. White *et al.* [2007] confirmed that the SNP within bovine *SPP1* gene successfully tracks functional alleles affecting growth in cattle. The previously undetected effect of SNP on birth and weaning weight of cattle [White *et al.* 2007] suggests that this particular *SPP1* marker may be a source of the phenotypic variance explained by QTL for birth weight, weaning weight and yearling weight on BTA-6.

The *SPP1 C>T* SNP marker investigated here was first reported by Leonard *et al.* [2005] in a trait-associated study with milk production. In a very recent report by Pareek *et al.* [2008], *SPP1 C>T* SNP polymorphism within the intron IV of bovine *SPP1* gene was investigated in selected breeds of beef (Hereford and Limousine) and dairy (Polish HF and Polish Red) cattle. Study revealed significant differences in the distribution of *C* and *T* alleles among the analysed panel of cattle breeds. Therefore, the objective of this study was to continue the evaluation of *SPP1 C>T* SNP polymorphism in the co-segregating HS families of growing Polish Holstein-Friesian cattle to confirm the association of bovine *SPP1* marker with body weight.

Material and methods

Animals

The Holstein-Friesian (HF) heifers (n=111) and young bulls (n=87) were used representing three half-sib (HS) families of sires heterozygous for *SPP1 C>T* SNP marker within the intron IV of *SPP1* gene. The distribution of heifers and bulls within three individual HS families was 22, 72, 17 and 31, 46, 10, respectively. The phenotypic parametre evaluated was body weight at the age of 3, 6 and 12 months within each HS family. The phenotypic information on body weight trait of 198 genotyped animals was obtained from commercial herds located at Kietrz and Krzyżanowo, Poland. All animals were fed the same commercially available feed and raised in the same common environment.

DNA isolation, polymorphism detection and genotyping

The source of DNA was venous blood. Genomic DNA was extracted from blood samples using Master Pure Genomic DNA purification kit (EPICENTER, USA). The following primers were used to investigate the bovine *SPP1* C>T SNP polymorphism [Leonard *et al.* 2005]:

SNP-8514 Intron-4 *SPP1* F: 5'-GCA AAT CAG AAG TGT GAT AGA C-3', and

SNP-8514 Intron-4 *SPP1* R: 5'-CCA AGC CAA ACG TAT GAG TT-3'.

Genotyping of bovine *SPP1* C>T SNP was carried out using PCR-RFLP procedure according to Leonard *et al.* [2005]. The PCR products (290 bp) were incubated with restriction enzyme *BsrI* (FERMENTAS, Lithuania) at 37°C for 3h. The enzyme distinguishes alleles C from alleles T of the SNP. The T allele represented as uncut fragment indicated by a band of 290 bp and the C allele represented by the bands of 200 bp and 90 bp (Photo 1).

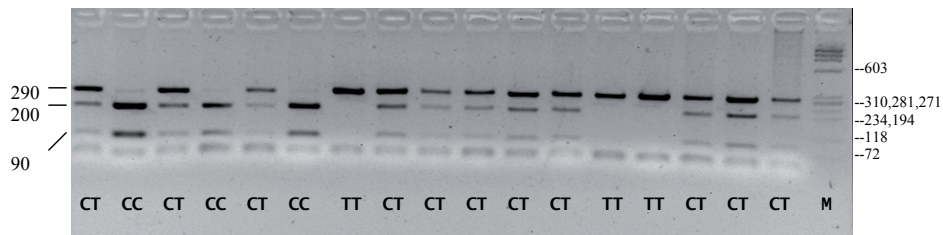


Photo 1. Bovine *SPP1* SNP C>T genotypic profile in the analysed HS progeny group of Polish HF cattle. On the left: allele bp size representing SNP genotypes, on the right: M = Φ X174 DNA/*HinfI*. On bottom: designated genotypes.

Statistical

The following linear model was fitted to 198 observations by the GLM procedure in SAS software 9.1 (SAS Institute, Inc., Cary, NC):

$$Y_{ijkl} = \mu + G_i + S_{ij} + S_{ijk} + e_{ijkl}$$

where:

Y_{ijkl} – the l-th observation of the dependent variable (body weight) for heifers, young bulls and both (pooled);

μ – overall mean;

G_i – effect of i-th *SPP1* genotypes (CC, CT, TT);

S_{ij} – fixed effect of j-th sire;

S_{ijk} – fixed effect of k-th sex (heifers and bulls);

e_{ijkl} – residual error.

Results and discussion

Body weight at the age of 3, 6 and 12 months was analysed for the association with the bovine *SPP1 C>T* SNP marker. Within the HS heifers the differences ($P \leq 0.05$) were found in the body weight at the age of 6 and 12 months between the bovine *SPP1* genotypes (8514C/C, 8514C/T and 8514T/T) – Table 1. However, within the HS group of young bulls such differences occurred ($P \leq 0.05$) between *SPP1* genotypes at the age of 3, 6 and 12 months (Tab. 2). Moreover, when data for sexes were pooled, the effects of *SPP1* genotypes on mean body weight in HS progeny of 6 and 12 months (Tab. 3) were found even highly significant ($P \leq 0.01$).

Table 1. Least squares means (LSM) and their standard errors (SE) for body weight of Polish Holstein-Friesian heifers aged 3, 6 and 12 months across *SPP1 C>T* SNP genotypes (three co-segregating HS families)

<i>SPP1 C>T</i> SNP genotype	n	Body weight (kg) at the age of					
		3 months		6 months		12 months	
		LSM	SE	LSM	SE	LSM	SE
C/C	28	103.1	2.56	169.8 ^a	4.04	308.9 ^a	5.30
C/T	56	103.2	1.87	178.9 ^b	2.95	318.3	3.87
T/T	27	105.5	2.72	179.5 ^b	4.29	322.4 ^b	5.63

^{ab}Means within columns bearing different superscripts differ significantly at $P \leq 0.05$.

Table 2. Least squares means (LSM) and their standard errors (SE) for body weight of Polish Holstein-Friesian bulls aged 3, 6 and 12 months across *SPP1 C>T* SNP genotypes (three co-segregating HS families)

<i>SPP1 C>T</i> SNP genotype	n	Body weight (kg) at the age of					
		3 months		6 months		12 months	
		LSM	SE	LSM	SE	LSM	SE
C/C	21	97.2 ^a	2.28	191.5 ^a	7.64	366.7 ^a	11.21
C/T	43	99.6	1.80	201.1	6.06	376.0	8.89
T/T	23	103.7 ^b	2.14	215.2 ^b	7.18	398.3 ^b	10.53

^{ab}Means within columns bearing different superscripts differ significantly at $P \leq 0.05$.

The results presented here reveal an association of co-segregating sires' allele *T* (favourable allele) with higher body weight in the HS progeny groups comprising young Polish HF heifers and bulls. Referring to production traits, the *SPP1 C>T* SNP genotypic frequencies were recently determined by Pareek *et al.* [2008] in young bulls of four beef and dairy breeds. Their study revealed that the frequency of favourable *T* allele was highest in Polish Red (67.4%), followed by Limousine (60.4%), Polish Holstein (55.4%) and Hereford (54.3%) bulls. Based on the cited study by Pareek *et al.* [2008] as well as on the present report, it could be suggested that *SPP1 C>T* SNP

Table 3. Least squares means (LSM) and their standard errors (SE) for body weight of Polish heifers and bulls (sexes pooled) aged 3, 6 and 12 months across *SPPI* C>T SNP genotypes (three co-segregating HS families)

<i>SPPI</i> C>T SNP genotype	n	Body weight (kg) at the age of					
		3 months		6 months		12 months	
		LSM	SE	LSM	SE	LSM	SE
C/C	49	100.9	1.78	181.2 ^{aa}	3.91	339.9 ^A	5.54
C/T	99	102.2	1.33	190.4 ^b	2.92	349.8	4.13
T/T	50	104.8	1.80	196.9 ^{bb}	3.94	360.5 ^B	5.59

^{aa}... Means within columns bearing different superscripts differ significantly at: small letters – P≤0.05; capitals – P≤0.01.

is a suitable candidate gene marker for higher body weight in growing animals also of other cattle breeds. However, such investigations are mainly handicapped because of lack of resource population among these breeds. In Poland, Polish HF and Polish Red cattle are also utilized for beef production. This study, therefore, leads to the conclusion that *SPPI* C>T SNP can be a marker of not only higher body weight (*i.e.* higher growth rate), but also of milk production in trait-associated studies on both breeds. Although in beef breeds the *SPPI* C>T SNP polymorphism was not, so far, investigated in trait-associated studies, an *in-del* polymorphism (T_{9}/T_{10}) within bovine *SPPI* gene was reported to be strongly associated with beef production traits [Allan *et al.* 2007, White *et al.* 2007]. Within dairy breeds, an association of C allele with increased fat and protein content of milk was reported by Leonard *et al.* [2005], while Khatib *et al.* [2007] showed significant effect of *SPPI* C>T SNP marker on milk fat (P≤0.0001) and milk protein (P≤0.0001) content, and on milk fat yield (P=0.014).

The results presented here show an association of bovine *SPPI* C>T SNP marker with higher body weight in co-segregating HS family of Polish HF heifers and young bulls. The favourable allele T showed significant effect on body weight in young bulls aged 3, 6 and 12 months and in 6 and 12 months aged heifers. Therefore, the investigated *SPPI* C>T SNP marker can be a suitable choice to simultaneous investigation of milk production traits (favouring C allele) and growth traits (favouring allele T) in large co-segregating HS families or other resource population of Polish HF cattle.

REFERENCES

1. ALLAN M.F., THALLMAN R.M., CUSHMAN R.A., ECHTERNKAMPS.E., WHITE S.N., KUEHN L.A., CASAS E., SMITH T. P. L., 2007 – Association of a single nucleotide polymorphism in SPP1 with growth traits and twinning in a cattle population selected for twinning rate. *Journal of Animal Science* 85, 341-347.
2. DENHARDT D.T., GUO X., 1993 – Osteopontin: a protein with diverse functions. *The FASEB Journal* 7, 1475-1482.
3. KHATIB H., ZAITOUN I., WIEBELHAUS-FINGER J., CHANG Y.M., ROSA G.J., 2007 – The association of bovine *PPARGC1A* and *OPN* genes with milk composition in two independent Holstein cattle populations. *Journal of Dairy Science* 90, 2966-2970.

4. LEONARD S., KHATIB H., SCHUTZKUS V., CHANG Y.M., MALTECCA C., 2005 – Effects of the osteopontin gene variants on milk production traits in dairy cattle. *Journal of Dairy Science* 88, 4083-4086.
5. PAREEK C.S., ZIĘBA M., MICHNO J., CZARNIK U., ZWIERZCHOWSKI L., 2008 – Study of SNP C>T polymorphism within the candidate genes for dairy and beef traits in a panel of selected cattle breeds. *Journal of Agro-biology* 25, 121-124.
6. SCHNABEL R.D., KIM J.J., ASHWELL M.S., SONSTEGAARD T.S., VAN TASSELL C.P., CONNOR E.E., TAYLOR J.F., 2005 – Fine-mapping milk production quantitative trait loci on BTA6. Analysis of the bovine osteopontin gene. *Proceedings of the National Academy of Sciences of the USA* 102, 6896-6901.
7. RANGASWAMI H., BULBULE A., KUNDU G.C., 2006 – Nuclear factor inducing kinase: a key regulator in osteopontin-induced MAPK/Ikappa kinase dependent NF-kappaB-mediated promatrix metalloproteinase-9 activation. *Trends in Cell Biology* 16, 79-87.
8. SENGER D.R., WIRTH D.F., HYNES R.O., 1979 – Transformed mammalian cells secrete specific proteins and phosphoproteins. *Cell* 16, 885-893.
9. WEINTRAUB A.S., LIN X., ITSKOVICH V.V., AGUINALDO J.G.S., CHAPLIN W.F., DENHARDT D.T., FAYAD Z.A., 2004 – Prenatal detection of embryo resorption in osteopontin-deficient mice using serial noninvasive magnetic resonance microscopy. *Pediatric Research* 55, 419-424.
10. WHITE S.N., CASAS E., ALLAN M.F., KEELE J.W., SNELLING W.M., WHEELER T.L., SHACKELFORD S.D., KOOHMARAIE M., SMITH T.P.L., 2007 – Evaluation in beef cattle of six deoxyribonucleic acid markers developed for dairy traits reveals an osteopontin polymorphism associated with postweaning growth. *Journal of Animal Science* 85, 1-10.

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Związek między polimorfizmem C/T w obrębie intronu IV genu osteopontyny a masą ciała rosnącego bydła rasy polskiej holsztyńsko-fryzyjskiej

Streszczenie

Analizowano możliwość wykorzystania polimorfizmu C/T w intronie IV genu osteopontyny (*SPP1*), jako czynnika różnicującego tempo wzrostu młodego bydła rasy polskiej holsztyńsko-fryzyjskiej (hf). Badaniami objęto jałówki (n=111) i młode buhaje (n=87), stanowiące grupy półrodzeństwa i będące potomstwem trzech buhajów o genotypie heterozygotycznym 8514C/T. Zwierzęta ważono w wieku 3, 6 i 12 miesięcy. Wykazano wpływ polimorfizmu SNP *SPP1* C>T na masę ciała jałówek, jak również młodych buhajów. Różnice (P≤0,05) w masie ciała między trzema grupami genotypowymi (8514C/C, 8514C/T i 8514 T/T) stwierdzono w przypadku 6- i 12-miesięcznych jałówek oraz 3-, 6- i 12-miesięcznych buhajów. Ponadto, analiza danych pochodzących od jałówek i buhajów (łącznie obie płci) wykazała wysoce istotny (P≤0,01) wpływ genotypu *SPP1* na masę ciała zwierząt 6- i 12-miesięcznych. W obu przypadkach wyższe wartości badanej cechy reprezentowały zwierzęta o genotypie 8514T/T. Autorzy wnioskują, że SNP *SPP1* C>T można traktować jako marker wielkości masy ciała młodego bydła hf.

