Short communication: Genetic association of variations in the osteopontin gene (*SPP1*) with lactation persistency in dairy cattle

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ABSTRACT

Improving lactation persistency (LP) in dairy cattle has a beneficial effect on animal health and fertility and herd productivity. A complex trait, LP not only reflects the cow's ability to maintain milk secretion activity after the lactation peak but is also a function of the postcalving development of the mammary gland and, later on, of tissue remodeling as lactation declines. This decline is a consequence of an imbalance between cell proliferation and cell removal. In a previous study, single nucleotide polymorphisms were identified in the osteopontin (OPN) gene, *SPP1*. Osteopontin is a multifaceted protein that plays an important role in immune regulation and tissue remodeling. Because OPN is involved in involution, it might also have an effect on LP. The objective of the present study was to evaluate whether LP could be influenced by genetic variations in the *SPP1* gene. This association with LP was analyzed in the population of 578 bulls characterized in a previous study. The population mean of estimated breeding value (EBV) for LP was 100.95 ± 5.06 units. Allele and genotype association analyses were performed by comparing the frequencies of the different genotypes and alleles with EBV for LP for the respective lactation using logistic regression. The EBV for LP at the first lactation (LP1), second lactation (LP2), and third lactation (LP3) and for overall lactation (OLP) are reported for the genotypes *SPP1c.-1301G>A*, *SPP1c.-1251C>T*, *SPP1c.-430G>A*, and *SPP1c.*40A>C*. The first single nucleotide polymorphism, *SPP1c.-1301G>A*, affected LP1, LP2, LP3, and OLP. Analysis of the estimated average allele substitution effects also confirmed that G is a favorable allele for LP, given the gain observed over LP1, LP2, LP3, and OLP. Differences in EBV for LP were observed between animals with different haplotypes at LP1, LP2, LP3, and OLP. Contrast analysis for OLP revealed that mean EBV is greater for block H1 (101.34 ± 0.30) than for animals that do not have

H1 (98.20 ± 0.77). The gain with block H1 (GCGA) suggests the presence of the favorable allele G (first position in the block: *SPP1c.-1301G*). The pleiotropic roles of OPN position it at the crossroads of immune regulation, tissue remodeling, and involution. From a genetic perspective, data from the present study suggest OPN as a candidate gene associated with LP for dairy cows.

Key words: lactation persistency, osteopontin, genetic association, dairy bovine

Short Communication

In dairy cattle, extended lactations have a beneficial effect on animal health and fertility and herd productivity. The lactation persistency (LP) trait is considered part of a strategy to decrease stressful peak yields associated with health problems while maintaining a high level of production after peak yield. The heritability of LP is moderate (0.18; Boettcher, 2005; Cole and VanRaden, 2006) to substantial (0.34, first lactation; Jamrozik et al., 2000). The EBV for LP, which have been available since 2000 in Canadian Holstein, is calculated for the first 3 lactations (Canadian Dairy Network, 2004). The LP can be computed based on solutions from the random regression test-day model (Jamrozik et al., 1998).

Few studies have investigated relationships between LP and health performance. Disease traits such as mastitis or SCS were found to confirm that LP deserves consideration in a breeding program (Cole and VanRaden, 2006; Appuhamy et al., 2007). Other health problems such as metabolic stress and negative energy balance were found to be negatively correlated with LP (Jakobsen et al., 2003; Walsh et al., 2011). On the whole, however, studies on extended lactation performance are scarce because LP is a complex trait. Lactation persistency not only reflects the ability to maintain milk secretion activity after the lactation peak but is also a function of postcalving mammary gland development and, later on, of tissue remodeling as lactation declines. This decline in milk yield after peak lactation in dairy animals reflects a decrease in the number of

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mammary cells, resulting from an imbalance between cell proliferation and cell removal (Stefanon et al., 2002; Capuco et al., 2003). Interestingly, in a genome-wide association study on LP, gene-enriched QTL showed that several networks related to cell development, proliferation, and death were enriched for LP positional candidate genes (Do et al., 2017). Numerous factors influence LP, notably hormonal changes (Stefanon et al., 2002; Yart et al., 2013, 2014) and cell regulation mechanisms leading to apoptosis (Sorensen et al., 2006; Norgaard et al., 2008). During involution, remodeling of mammary tissue occurs (Herve et al., 2016). This process is characterized by extensive cell death and the acquisition of an inflammatory profile. These events have been described as the signature of wound healing, including an influx of macrophages that support tissue remodeling (O'Brien et al., 2012).

The protein osteopontin (**OPN**) is secreted in numerous body fluids, including blood and milk. This multifaceted protein plays an important role in tissue homeostasis and remodeling, immune regulation, and stress response (Denhardt and Noda, 1998; Denhardt et al., 2001; Sodek et al., 2006; Wang and Denhardt, 2008), and also contributes to macrophage homing and cellular immunity (Ashkar et al., 2000). Expression of the OPN gene (*SPP1*) increases locally in the bovine mammary gland during bacterial infection to improve cell-mediated immunity (Denhardt et al., 2001; Konno et al., 2006; Alain et al., 2009). Osteopontin is naturally present in bovine milk, and interestingly, genetic variations of a cow *SPP1* influence the amount of OPN secreted in its milk throughout lactation (Dudemaine et al., 2014). The gene *SPP1* is part of the molecular mechanisms involved during involution of the mammary gland in mice (Baik et al., 1998; Prokesch et al., 2014). The hypothesis of the present study is that OPN may also affect LP because of its role in involution.

In a previous study, 4 SNP in *SPP1* were identified to be associated with the mammary health status of dairy cows (Alain et al., 2009). These SNP, namely, *SPP1c.-1301G>A* (rs109637038), *SPP1c.-1251C>T* (rs109637038), *SPP1c.-430G>A* (rs108997065), and *SPP1c.*40A>C* (rs132812135), were identified by sequencing the promoter, exons, and 3' untranslated region of animals with extreme EBV for SCS (Alain et al., 2009). From these SNP, the haplotype blocks H1 to H5 were defined. The analysis of the functional effect of the haplotypes confirmed that genetic variations in the *SPP1* promoter affect the level of OPN secretion into bovine milk (Dudemaine et al., 2014). In addition, immune cells isolated from cows with different haplotypes differentially respond to bacterial ex vivo infection. As these SNP affect gene expression, they may account

Table 1. Genotypes and haplotype frequencies of the 578 bull population

Genotype ¹				Frequency	%
Allele 1		Allele 2			
H1	(GCGA)	H1	(GCGA)	205	35.5
H1	(GCGA)	H2	(GTAC)	170	29.4
H1	(GCGA)	H3	(ACGA)	76	13.1
H1	(GCGA)	H4	(GCGC)	30	5.2
H2	(GTAC)	H2	(GTAC)	37	6.4
H2	(GTAC)	H3	(ACGA)	28	4.8
H2	(GTAC)	H4	(GCGC)	13	2.2
H3	(ACGA)	H3	(ACGA)	6	1.0
H3	(ACGA)	H4	(GCGC)	5	0.9
H4	(GCGC)	H4	(GCGC)	2	0.3
H4	(GCGC)	H5	(GTAA)	6	1.0
Total				578	100

¹H1–H4 = haplotype blocks 1 to 4.

for phenotypic variation of milk production and health traits (Dudemaine et al., 2014). The objective of the present study was to evaluate whether LP could be influenced by genetic variations in the *SPP1* gene. The association of these SNP with LP was therefore analyzed in the population of 578 bulls characterized in a previous study (Alain et al., 2009). The genotypes and haplotype frequencies of the 4 SNP identified among the 578 bulls are summarized in Table 1.

For the Holsteins breed, bull proofs for LP reflect the average daughter milk yield at lactation d 280 compared with lactation d 60. The scale used is standardized so that, in the base population, the calculated weighted percentage of EBV for LP has a mean of 100 and a standard deviation of 5. As a result, LP is an arbitrary relative value with no defined units. The EBV of LP for the 578 bulls were provided by the Canadian Dairy Network (Guelph, ON, Canada; <https://www.cdn.ca/>). The mean for the EBV of LP of the 578 bulls was 100.95 ± 5.06 units. Allele and genotype association analyses were performed by comparing the frequencies of the different genotypes and alleles with EBV for LP for the respective lactation. Data were analyzed using the SAS software (release 9.4, SAS Institute Inc., Cary, NC). The model for either genotype or haplotype effect was a one-way factorial with unequal variances due to the strongly unbalanced number of animals per group. When a genotype effect was found significant, a Tukey adjusted multiple comparisons was further performed. The EBV for LP at the first lactation (**LP1**), second lactation (**LP2**), and third lactation (**LP3**) and for overall lactation (**OLP**) are reported in Table 2 for genotypes *SPP1c.-1301G>A*, *SPP1c.-1251C>T*, *SPP1c.-430G>A*, and *SPP1c.*40A>C*. The first SNP, *SPP1c.-1301G>A*, affected LP1, LP2, LP3, and OLP ($P < 0.001$). The estimated haplotype frequencies of

Table 2. Effects of SNP in the bovine osteopontin gene (*SPP1*) on EBV for lactation persistency for overall lactation (OLP) and for the first lactation (LP1), second lactation (LP2), and third lactation (LP3)¹

SNP ²	LSM ± SEM ³			P-value
	GG	GA	AA	
<i>SPP1c.-1301G>A</i>				
OLP	101.40 ± 0.23 ^a	99.38 ± 0.48 ^b	94.83 ± 2.03 ^b	<0.001
LP1	100.89 ± 0.23 ^a	99.03 ± 0.47 ^b	95.50 ± 2.02 ^b	<0.001
LP2	101.62 ± 0.24 ^a	99.51 ± 0.48 ^b	96.50 ± 2.06 ^b	<0.001
LP3	101.36 ± 0.23 ^a	99.84 ± 0.47 ^b	94.67 ± 1.99 ^c	<0.001
<i>SPP1c.-1251C>T</i>				
	CC	CT	TT	
OLP	101.00 ± 0.28	100.98 ± 0.34	100.32 ± 0.83	0.738
LP1	100.53 ± 0.28	100.48 ± 0.34	100.08 ± 0.83	0.875
LP2	101.27 ± 0.29	101.11 ± 0.35	100.65 ± 0.84	0.768
LP3	101.16 ± 0.28	100.91 ± 0.34	100.16 ± 0.81	0.479
<i>SPP1c.-430G>A</i>				
	AA	GA	GG	
OLP	100.32 ± 0.83	100.98 ± 0.34	101.00 ± 0.28	0.738
LP1	100.08 ± 0.83	100.48 ± 0.34	100.53 ± 0.28	0.875
LP2	100.65 ± 0.84	101.11 ± 0.35	101.27 ± 0.29	0.768
LP3	100.16 ± 0.81	100.91 ± 0.34	101.16 ± 0.28	0.479
<i>SPP1c.*40A>C</i>				
	AA	AC	CC	
OLP	100.95 ± 0.30	101.16 ± 0.33	100.02 ± 0.70	0.339
LP1	100.56 ± 0.30	100.55 ± 0.33	99.77 ± 0.70	0.561
LP2	101.17 ± 0.30	101.34 ± 0.33	100.39 ± 0.71	0.481
LP3	101.03 ± 0.29	101.19 ± 0.32	100.00 ± 0.69	0.289

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).
¹The 3 individual lactation proofs using EBV for persistency are combined to produce the overall lactation persistency (OLP): the emphasis, 50% of EBV, is placed on the first lactation (LP1), whereas the second lactation persistency (LP2) and third lactation persistency (LP3) each receive a weight of 25% (Canadian Dairy Network, 2004).
²*SPP1c.-1301G>A* refers to rs109637038, *SPP1c.-1251C>T* refers to rs109637038, *SPP1c.-430G>A* refers to rs108997065, and *SPP1c.*40A>C* refers to rs132812135.
³The LSM are the adjusted means of the EBV for lactation persistency for bulls, which are grouped by genotype for each SNP and calculated for the respective lactation.

the 578 bulls showed previously that 5 haplotypes (H1 to H5) represent 99.2% of the alleles in this population (Alain et al., 2009). Differences were also observed between haplotype groups with EBV for LP at LP1 ($P = 0.001$), LP2 ($P = 0.001$), LP3 ($P = 0.002$), and OLP ($P < 0.001$; Table 3).

The average allele substitution effects of the SNP were calculated using the following model also described in Leyva-Baca et al. (2008):

$$Y_{jk} = \mu + \sum_{i=1}^4 \beta_i G_i + S_j + e_{jk},$$

where y_{jk} = trait EBV for the j th bull from the k th sire, μ = overall mean, β_i = fixed regression coefficient for the i th SNP, G_i = the genotype of the i th SNP recoded, as described elsewhere (Zeng et al., 2005), as follows:

$$G_i = \begin{cases} -1 & \text{for homozygous genotype 1} \\ 0 & \text{for heterozygous} \\ +1 & \text{for homozygous genotype 2} \end{cases},$$

S_j = random polygenic effect of the j th sire ($j = 1$ to 26), and e_{jk} = random error.

This analysis confirmed that G is a favorable allele for LP, given the gain observed over LP1 ($P = 0.007$), LP2 ($P = 0.013$), LP3 ($P = 0.050$), and OLP ($P = 0.009$; Table 4). No deregression of the EBV for LP with the parent average removed was applied to generate higher reliabilities of genomic breeding values. It might have improved haplotype substitution effects, which was not found to be significant (data not shown). However, the difference was significant at a comparison-wise level with EBV for LP. Indeed, contrast analysis for OLP

Table 3. Haplotype effects of the bovine osteopontin gene (*SPP1*) on EBV for lactation persistency for overall lactation (OLP) and for the first lactation (LP1), second lactation (LP2), and third lactation (LP3)¹

Item	EBV units of animals classified based on their haplotype																SEM	P-value
	H1 (GCGA)	H1 (GCGA)	H1 (GCGA)	H1 (GCGA)	H1 (GCGA)	H1 (GCGA)	H1 (GCGA)	H1 (GCGA)	H1 (GCGA)	H1 (GCGA)	H1 (GCGA)	H1 (GCGA)	H1 (GCGA)	H1 (GCGA)	H1 (GCGA)	H1 (GCGA)		
	H1 (GCGA)	H2 (GTAC)	H3 (ACGA)	H4 (GCGC)	H2 (GTAC)	H3 (ACGA)	H4 (GCGC)	H2 (GTAC)	H3 (ACGA)	H4 (GCGC)	H2 (GTAC)	H3 (ACGA)	H4 (GCGC)	H2 (GTAC)	H3 (ACGA)	H4 (GCGC)		
OLP	101.48	101.69	100.01	102.17	100.32	97.79	99.54	94.83	95.50	98.60	97.50	98.83	97.17	98.83	97.50	98.83	3.50	<0.001
LP1	101.07	101.14	99.58	100.97	100.08	97.71	99.31	95.50	96.50	98.00	97.00	97.17	97.00	98.83	97.00	97.17	3.49	0.001
LP2	101.70	101.77	100.11	102.87	100.65	98.00	100.15	96.50	96.50	99.00	97.00	98.83	97.00	98.83	97.00	98.83	3.56	0.001
LP3	101.48	101.41	100.34	102.80	100.16	98.50	99.69	94.67	94.67	99.60	99.00	100.67	99.00	100.67	99.00	100.67	3.44	0.002

¹The 3 individual lactation proofs using EBV for persistency are combined to produce the overall lactation persistency (OLP); the emphasis, 50% of EBV, is placed on the first lactation (LP1), whereas the second lactation persistency (LP2) and third lactation persistency (LP3) each receive a weight of 25% (Canadian Dairy Network, 2004).

revealed that mean EBV is greater ($P < 0.001$) for block H1 (101.34 ± 0.30) than for animals that do not have H1 (98.20 ± 0.77). The 1.98% gain with block H1 (GCGA) suggests a positive effect on the presence of allele G (first position in the block: allele *SPP1c.-1301G*). In a previous study (Dudemaine et al., 2014), OPN secretion was quantified in milk throughout lactation for 2 groups of cows harboring haplotypes H1-GCGA $\times$ H4-GCGC ($n = 5$) and H2-GTAC $\times$ H3-ACGA ($n = 5$). Marked increases in both concentration and total quantity of OPN were found for H1 $\times$ H4 cows in comparison with H2 $\times$ H3 cows starting in wk 25 of lactation (Dudemaine et al., 2014). The H1 and H4 haplotypes contain the same promoter segment (the SNP GCG). Using functional analysis of the promoter of *SPP1*, it was shown that the activity of the segment H1-GC was greater ($P < 0.001$) than the activity of H2 and H3 (Dudemaine et al., 2014). Interestingly, in the present study, contrast analysis performed for LP for these haplotype groups (i.e., H1 $\times$ H4 vs. H2 $\times$ H3) also confirmed a difference for LP1 ($P = 0.012$), LP2 ($P < 0.001$), LP3 ($P < 0.001$), and OLP ($P < 0.001$). However, this gain of 1.98% for OLP obviously cannot be attributed only to *SPP1c.-1301G* because other haplotypes (i.e., H2, H4, and H5) also carry this genotype without having a similar beneficial effect on LP. Considering that the 4 SNP from this analysis were identified in a population selected based on extreme EBV for SCC, other genetic variations could have been identified for the LP trait using bulls having extreme EBV for LP. Thus, sequencing the exons, introns, and promoter region of those bulls having extreme EBV for LP would enable identification of additional SNP with *SPP1c.-1301G* and provide a better understanding of the molecular map of genetic variations associated with LP. Notwithstanding the missing pieces of the puzzle, a gain of $\sim 2\%$ of LP is an important economic benefit that can be attributed to numerous factors, including milk production yields, metabolic disease, and reproductive performance. Higher 305-d yields and greater LP were associated with a longer calving interval (Muir et al., 2004); together, these factors have a positive effect on longevity (Walker, 2014; Nayeri et al., 2017). The numerous factors affecting LP make it difficult to identify the source of the $\sim 2\%$ gain and therefore to quantify the economic gain.

From a molecular perspective, variation at the position of the SNP *SPP1c.-1301G>A* can potentially affect 2 recognition motifs: the mammalian transcriptional repressor RBP-J κ /CBF1 and the Ikaros transcription factor (IKZF1/2). In silico analysis of both alleles using the MatInspector search tool from the Genomatix software suite (www.genomatix.de) suggests that *SPP1c.-1301G* abolishes both the RBP-J κ /

Table 4. Estimated genotype effects on EBV for lactation persistency for overall lactation (OLP) and for the first lactation (LP1), second lactation (LP2), and third lactation (LP3)¹

SNP ²	Average genotype substitution effect in EBV units			
	OLP	LP1	LP2	LP3
<i>SPP1c.-1301G>A</i>	-1.23 ± 0.47 (0.009)	-1.31 ± 0.49 (0.007)	-1.17 ± 0.47 (0.013)	-0.92 ± 0.47 (0.050)
<i>SPP1c.-1251C>T</i>	0.06 ± 0.74 (0.936)	0.18 ± 0.76 (0.812)	-0.24 ± 0.74 (0.744)	-0.18 ± 0.73 (0.808)
<i>SPP1c.-430G>A</i>	-0.06 ± 0.74 (0.936)	-0.18 ± 0.76 (0.812)	0.24 ± 0.74 (0.744)	0.18 ± 0.73 (0.808)
<i>SPP1c.*40A>C</i>	-0.25 ± 0.70 (0.718)	-0.35 ± 0.72 (0.628)	0.00 ± 0.70 (0.998)	-0.14 ± 0.70 (0.839)

¹The 3 individual lactation proofs using EBV for persistency are combined to produce the overall lactation persistency (OLP): the emphasis, 50% of EBV, is placed on the first lactation (LP1), whereas the second lactation persistency (LP2) and third lactation persistency (LP3) each receive a weight of 25% (Canadian Dairy Network, 2004). Values are presented as estimated genotype effect ± SEM (*P*-value).

²*SPP1c.-1301G>A* refers to rs109637038, *SPP1c.-1251C>T* refers to rs109637038, *SPP1c.-430G>A* refers to rs108997065, and *SPP1c.*40A>C* refers to rs132812135.

CBF1 and IKZF1/2 binding motifs but *SPP1c.-1301A* does not (data not shown). The motif IKZF1/2 was also identified in a previous study (Alain et al., 2009) as being associated with the allele *SPP1c.-1301A*, using the software MOTIF Search (<http://motif.genome.jp/>). Ikaros appears to exert its influence on chromatin remodeling through the induction of epigenetic changes that are characteristic of transcriptionally repressed chromatin (Gowda et al., 2016; Wang et al., 2016). Interestingly, the second DNA-binding factor, RBP-J, is a methyl-CpG-binding protein (Bartels et al., 2011) and thus also has the capacity to repress the activity of chromatin through epigenetic modifications (Schwanbeck, 2015). Moreover, RBP-J was previously found to switch off target gene expression and ensure the tight spatiotemporal control of the gene expression response of the target gene (Liefke et al., 2010). Although the interaction of Ikaros or RBP-J with the promoter of *SPP1* has not been confirmed, the genetic analysis deserves consideration because of the *SPP1c.-1301G>A* functional marker. From a genetic perspective, it is well known that health and disease may affect other performance traits such as longevity and LP. Osteopontin is a multifaceted protein at the crossroads of immune regulation, wound healing, tissue remodeling, and involution. Data from the present study suggest OPN as a candidate gene associated with LP for dairy cows.

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