J Appl Genetics (2015) 56:505–513
DOI 10.1007/s13353-015-0278-5

ANIMAL GENETICS • ORIGINAL PAPER

Single nucleotide polymorphisms in candidate genes and their relation with somatic cell scores in Argentinean dairy cattle

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Received: 6 March 2014 / Revised: 19 January 2015 / Accepted: 10 February 2015 / Published online: 18 March 2015

Abstract The prevention and control of bovine mastitis by enhancing natural defenses in animals is important to improve the quality of dairy products. Mastitis resistance is a complex trait which depends on genetic components, as well as environmental and physiological factors. The limitations of classical control measures have led to the search for alternative approaches to minimize the use of antibiotics by selecting naturally resistant animals. Polymorphisms in genes associated with the innate immune system are strong candidates to be evaluated as genetic markers. In this work, we evaluated a set of single nucleotide polymorphisms (SNPs) in candidate genes for health and production traits, and determined their association with the somatic cell score (SCS) as an indicator of mastitis in Argentinean dairy cattle. We evaluated 941 cows: Holstein (n=677) and Holstein × Jersey (n=264) crossbred, daughters from 22 bulls from 14 dairy farms located in the central dairy area of Argentina. Two of the 21 successfully genotyped markers were found to be significantly associated (p<0.05) with the SCS: GHR_140 and OPN_8514C-T. The heterozygote genotype for GHR_140 showed a favorable effect in reducing the SCS. On the other hand, heterozygote genotypes for OPN8514C-T caused an increase in the SCS; moreover, combined genotypes for OPN SNPs showed an even larger effect. These findings can contribute to the design of effective marker-assisted selection programs.

Keywords Mastitis · Resistance · Polymorphisms · Milk

Communicated by: Maciej Szydlowski

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Introduction

Bovine mastitis is defined as an inflammatory condition of the mammary gland in response to injury directed to neutralize and eliminate infectious agents, promote healing, and return to normal function (Bramley et al. 1996). Economic losses associated with this disease are attributed to reduced milk yield and quality, reduced lactation persistency, and early culling (Seegers et al. 2003; Capuco et al. 2003). Mastitis control is based on milking-time hygiene, lactational and dry cow antibiotic therapy, periodic maintenance of the milking machine, and culling of chronically infected cows (Bramley et al. 1996). Despite these practices, the limited cure of *Staphylococcus aureus* intramammary infections (IMIs) following antibiotic therapy (Zecconi et al. 2006) and the lack of dry cow antibiotic therapeutic levels during the periparturient period against environmental pathogens (Todhunter et al. 1991) make this disease difficult to control. In addition, indiscriminate use of antibiotics could promote the emergence of bacterial strains resistant to antibiotics. These limitations have led to the search for alternatives approaches in order to minimize the use of antibiotics, both by stimulating the animal's defensive systems to control infections and by selecting animals showing greater natural resistance to IMIs (Denis et al. 2009; Rupp and Boichard 2003). The prevention and control of mastitis by enhancing natural defenses in animals is not only important to improve the quality of dairy products, but also to decrease

consumers' concerns about issues such as food security and animal welfare (Rupp et al. 2007). Subclinical mastitis is indirectly diagnosed by the measurement of the somatic cell count (SCC) in milk, where an increase in the SCC is mainly due to the influx of white blood cells from blood to milk directed to eliminate infectious agents from the mammary gland. The SCC is positively correlated with the inflammatory response of the mammary gland, presenting higher heritability than clinical mastitis (Lund et al. 1999) and is highly correlated with bacterial infections (Weller et al. 1992). This parameter has been extensively used for the genetic improvement of resistance to mastitis (Shook and Schutz 1994; Heringstad et al. 2000).

Mastitis resistance is a complex trait which depends on genetic components, as well as environmental and physiological factors. Resistance can be defined as the cow's ability to prevent or to quickly overcome infection. Many chromosomal regions have been associated with resistance to mastitis (http://www.animalgenome.org/cgi-bin/QTLdb/BT/index). The search for identifying genetic markers associated with resistance to mastitis has focused on two approaches: the search for quantitative trait loci (QTL) and single nucleotide polymorphisms (SNPs) in candidate genes. The candidate gene approach assumes that a gene involved in the physiology of the trait may contain a mutation causing a variation in phenotype. The gene, or part of the gene, is sequenced in different animals and variations in the DNA sequence (polymorphisms) are statistically tested for association with variation in the phenotype (Hayes and Goddard 2010). With a more focused approach, this process can identify additional markers suitable for marker-assisted selection on experimental models. Polymorphisms in genes associated with the innate immune system are strong candidates to be evaluated in this type of study. Genes encoding receptors and factors responsible for detecting and eliminating pathogens are likely to contribute to the overall variation in resistance or susceptibility to mastitis in dairy cattle (Sharma et al. 2011). Several studies have identified polymorphisms in genes encoding the bovine major histocompatibility complex (bovine leukocyte antigen; BoLA) (Rupp et al. 2007; Sharif et al. 1998) and cytokine receptors (Youngerman et al. 2004). Also, gene polymorphisms related to innate immune responses, such as lactoferrin, have been studied (Wojdak-Maksymiec et al. 2006). Other types of candidates are the positional candidates, genes located in a genomic region identified from a genome scan as being likely to host a QTL. Many QTLs associated with production traits have been shown to be closely related to mastitis, as there is a well-established negative correlation between production and mastitis (Oltenacu and Broom 2010). Another way to obtain candidates genes is to analyze metabolic pathways and genes involved in the immune response that show differential expression patterns using microarrays on experimentally infected animals (Lutzow et al. 2008; Swanson et al. 2009). The aim of this work was to evaluate a set of recently identified SNPs in candidate genes and determine their association with the log transformation of the SCC, referred to as the somatic cell score (SCS), as an indicator of mastitis in Argentinean dairy cattle.

Materials and methods

Animals and phenotypic data

The study included a total of 6,584 records from 941 cows: Holstein (n=677) and Holstein × Jersey (n=264) crossbred. Crosses were up to 87.5 % Jersey, the average being 87 % Holstein. All cows were daughters from 22 bulls and belonged to 14 dairy farms of only one dairy industry, located within 10 km from each other, in the province of Santa Fe, Argentina. All animals were kept in the same feeding and sanitary management and were machine milked twice a day. The SCC was recorded every 40 days by an official DHI.

For SCC determination, milk samples were preserved with azidiol (0.3 %) at 4 °C and analyzed within 24 h. The SCC determinations were performed by a commercial laboratory (Laboratorio Regional de Servicios Analíticos, Esperanza) using an automated counter, Somacount 300 (Bentley Instruments, Chaska, MN, USA). Milk samples were warmed up at 39–40 °C to facilitate homogenization. All of the studied cows had at least four records during one lactation (the average was seven). The analyzed data corresponded to 20 to 400 days in milk.

DNA extraction

Bovine blood samples were collected from the jugular veins into tubes containing EDTA as the anticoagulant. Genomic DNA was extracted from fresh blood using a commercial kit (AxyPrep Blood Genomic DNA Miniprep Kit, Axygen Biosciences, Union City, CA, USA), according to the protocol provided by the manufacturer. The quantity and quality of DNA were checked by measuring the absorbance in a Thermo Scientific NanoDrop™ 1000 Spectrophotometer.

Selection of candidate gene markers

The markers were selected from different sources: (i) SNPs from genes that belonged to QTL mapping studies from QTLdb (http://www.animalgenome.org/QTLdb/cattle.html); (ii) SNPs in genes previously associated with production and disease resistance traits; (iii) SNPs in differentially expressed genes in transcriptional studies. Databases such as dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) and Ensembl (http://www.ensembl.org) were used to locate the position and flanking sequence of each marker.

Genotyping

Genotyping was performed using the SNPlex Genotyping System (Applied Biosystems) (Tobler et al. 2005). A genotyping panel of 40 SNPs was designed using the manufacturer pipeline for SNPs within 18 candidate genes and regions. Three of the SNPs were discovered by our group by the sequencing of selected gene regions in the population sires (unpublished). Quality control of the results was done using GeneMapper v4 software (Applied Biosystems).

Statistical analysis

Genotypic and allele frequencies for each SNP were calculated, and the Chi-square test was used to verify that allele segregation was in agreement with Hardy–Weinberg equilibrium (HWE). Association analysis between genotypes and the SCS (SCS= log2 [SCC/100,000)+3]) was carried out using linear mixed models fitted by restricted maximum likelihood estimation using WOMBAT (Meyer 2007) applying the following model:

$$Y_{ijklmno} = \mu + L_i + HYST_j + DIM_k + HF_l + G_m + A_n + PE_o + e_{ijklmno}$$

where $Y_{ijklmno}$ is the test day record of the SCS; μ is the herd overall SCS mean; L_i is the fixed effect of lactation number; $HYST_j$ is the fixed effect for the combination of herd (H), year (Y), and season (S) of the test record; DIM_k is the fixed covariate of days in milk; HF_l is the fixed effect of the proportion of Holstein blood in the animal; G_m is the fixed effect of the genotype/combined genotype for each SNP; A_n is the random additive genetic effect of the cow distributed as N(0, Aσ_a^2), which accounted for (co)variances between animals due to genetic relationships by the formation of a matrix A based on pedigree records; PE_o is the random permanent environmental effect distributed as N(0, Iσ_{pe}^2); and $e_{ijklmno}$ is the random error distributed as (0, Iσ_e^2). The likelihood and Akaike's information criterion were assessed (Akaike 1973) to determine the relative quality of the model with the included effects. To account for the risk of false-positives, a false discovery rate (FDR) was used. The *p*-value threshold corresponding to an FDR of 0.20 was calculated using the package *qvalue* in R (Storey and Tibshirani 2003). SNPs with *p*-values less than or equal to the *q*-value corresponding to the FDR were considered significantly associated.

Heritabilities were calculated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2}$$

where σ_a^2 represents the estimated additive genetic variance; σ_{pe}^2 is the estimated permanent environment variance; and σ_e^2 is the residual variance.

Results

SNPs markers

From the total of 40 SNPs that were included in the panel, 38 passed the quality control in GeneMapper v4 of signal median and cluster discrimination. The two discarded SNPs were located within the interleukin 8 (IL8) and fatty acid synthase-1 (FASN-1) genes. Three additional SNPs were removed because they were beyond the scope of this work, since they are the causative mutation of genetic disorders: BLAD (bovine leukocyte adhesion deficiency; Shuster et al. 1992), DUMPS (deficiency of uridine monophosphate synthase; Schwenger et al. 1993), and CVM (complex vertebral malformation; Thomsen et al. 2006). Of the 35 remaining SNPs, 6 (17 %) were removed because of a poor genotype call rate (below 90 %). Those SNPs were located on lactoglobulin (LGB; three SNPs), alpha-S1 casein (CSN1), oxidized low density lipoprotein receptor-1 (OLR1), and signal transducer and activator of transcription 5A (STAT5) genes. In conclusion, after filtering SNPs based on the calling rate and SNPlex® platform quality rules, the genotypes for 29 SNPs were analyzed. Minor allele frequencies (MAF) for the analyzed SNPs are presented in Table 1. From the 29 SNPs remaining, three of them were monomorphic for this selected group of animals, so they were excluded from further analysis. One of the SNPs, ABCG2_199 [within the ATP-binding cassette, subfamily G (WHITE), member 2 gene], was then removed because its MAF did not reach the 0.05 limit. Then, as a genotyping quality control, the Hardy–Weinberg test was performed. Of the 25 remaining markers, four showed significant deviations from HWE and were also discarded. Finally, the 21 remaining SNPs were analyzed to find associations. Three of them, PRL_in1 (SNP in bovine prolactin gene, intron 1), GH_51, and GH_335 (SNPs within the growth hormone gene), were newly discovered by sequencing each selected gene region (unpublished). The MAF from the final set of 21 SNPs ranged from 0.08 for PPARGC1A_337 (peroxisome proliferator-activated receptor-γ coactivator 1α gene) to 0.48 for GH_51.

Association analysis

After the analyses, two of the 21 successfully genotyped markers were found to be significantly associated ($p<0.05$) with the SCS. The results of the analysis are shown in Table 2. These two SNPs, GHR_140 (within the growth hormone receptor gene) and OPN_8514C-T (osteopontin, or secreted phosphoprotein 1 gene, SPP1), were previously described as being associated with milk traits. GHR_140 is a substitution of an A for a T in exon 8 of the growth hormone receptor gene (GHR), leading to phenylalanine for tyrosine change at position 279 (F279Y), and it is located within the transmembrane

Table 1 Summary statistics for the genotyped single nucleotide polymorphisms (SNPs)

SNP name	rs identifier or amino acid change	Gene	Gene location	SNP location Chr:nucleotide	Genotype	Genotypic frequency	MAF	HWE
SNP9	rs43375517	ARL4A	5′UTR	4:20,296,999	C/C	0.10	0.34	0.21
					G/C	0.47		
					G/G	0.43		
SNP12	rs42213673	ETV1	Intergenic	4: 22,111,819	C/C	0.41	0.36	0.52
					C/T	0.47		
					T/T	0.12		
SNP24	rs43745957		Intergenic	4:24,305,381	C/C	0.22	0.45	0.16
					C/T	0.47		
					T/T	0.31		
OBESE_305T-C	rs29004488	OBESE-LEP	Exon	4:93,262,056	C/C	0.28	0.45	0.00**
					C/T	0.54		
					T/T	0.18		
ABCG2_199	rs43702337	ABCG2	Exon	6:38,027,010	A/A	0.98	0.01*	0.81
					A/C	0.02		
					C/C	0.00		
SPP1_11740	rs132812135	SPP1/OPN	3′UTR	6:38,120,968	C/C	0.08	0.29	0.87
					A/C	0.41		
					A/A	0.51		
OPN_8514C-T	Intron 4 (position 8514 NW_255516.1)	SPP1/OPN	Intron	6:38,122,666	C/C	0.28	0.47	0.80
					C/T	0.50		
					T/T	0.22		
PPARGC1A_c1892	rs109579682	PPARGC1A	Intron	6:44,875,251	A/A	0.04	0.21	0.57
					A/G	0.34		
					G/G	0.62		
PPARGC1A_230	rs133669403	PPARGC1A	Exon	6:44,875,315	A/A	0.02	0.14	0.27
					A/G	0.25		
					G/G	0.74		
PPARGC1A_337	rs17870811	PPARGC1A	Intron	6:44,875,421	C/C	0.85	0.08	0.21
					C/T	0.15		
					T/T	0.00		
CSN1_BD	A53T	CSN1	Exon	6:87,148,464	A/A	0.00	0.00*	0.00
					A/G	0.00		
					G/G	1.00		
CSN1_BF	S66L	CSN1	Exon	6:87,150,003	C/C	1.00	0.00*	0.00
					C/T	0.00		
					T/T	0.00		
CSN1_BCF	rs43703010	CSN1	Exon	6:87,157,262	A/A	0.81	0.10	0.93
					A/G	0.18		
					G/G	0.01		
CSN2_Ser122Arg	rs43703013	CSN2	Exon	6:87,181,453	C/C	0.90	0.06	0.00**
					G/C	0.09		
					G/G	0.01		
CSN2_Pro67His	rs43703011	CSN2	Exon	6:87,181,619	C/C	0.40	0.35	0.02**
					A/C	0.49		
					A/A	0.11		
CSN3_68	rs43706475	CSN3	Exon	6:87,390,479	T/T	1.00	0.00*	0.00
					T/G	0.00		
					G/G	0.00		
CSN3_165	rs43703015	CSN3	Exon	6:87,390,576	C/C	0.72	0.15	0.12
					C/T	0.26		
					T/T	0.01		
CSN3_221	rs43703017	CSN3	Exon	6:87,390,632	A/A	0.83	0.09	0.37
					A/G	0.17		
					G/G	0.01		
IL8_28	rs43707839	IL8	Splice	6:90,561,773	C/C	0.25	0.50	0.90
					A/C	0.50		
					A/A	0.26		
GH_335	Unpublished	GH	Exon	19:48,768,766	C/C	0.03	0.14	0.01**
					A/C	0.22		
					A/A	0.75		
GH_51	Unpublished	GH	Intron	19:48,769,040	C/C	0.27	0.48	0.58
					C/T	0.51		
					T/T	0.22		

Table 1 (continued)

SNP name	rs identifier or amino acid change	Gene	Gene location	SNP location Chr:nucleotide	Genotype	Genotypic frequency	MAF	HWE
GHR_140	F279Y	GHR	Exon	20:31,909,479	T/T	0.70	0.16	0.17
					T/A	0.26		
					A/A	0.03		
PRLR_S18N	rs135164815	PRLR	Exon	20:39,115,344	A/A	0.73	0.14	0.57
					G/A	0.25		
					G/G	0.02		
UTMP_1296G-A	rs132991801	UTMP	Exon	21:59,667,572	A/A	0.13	0.36	0.80
					A/G	0.47		
					G/G	0.40		
LTF_90	rs41256920	LTF	5′UTR	22:53,521,978	C/C	0.05	0.22	0.71
					C/A	0.35		
					A/A	0.61		
LTF_250	rs43706485	LTF	5′UTR	22:53,522,038	C/C	0.04	0.21	0.90
					C/G	0.33		
					G/G	0.63		
PRL_g8398G-A	rs211032652	PRL	Exon	23:35,106,206	A/A	0.01	0.12	0.80
					A/G	0.21		
					G/G	0.78		
PRL_in1	Unpublished	PRL	Exon	23:35,110,940	C/C	0.08	0.30	0.09
					C/T	0.44		
					T/T	0.48		
SCD1_A293V	rs41255693	SCD1	Splice site	26:21,144,707	C/C	0.72	0.15	0.33
					C/T	0.25		
					T/T	0.03		

Positions correspond to the UMD3.1 bovine genome assembly. For SNPs without an rs identifier, the published notation or protein change was used to identify them. Genotype and allele frequencies and the significance of deviations from Hardy–Weinberg equilibrium (*HWE*) based on *p*-values obtained from the Chi-square test results are shown. All SNP nucleotide positions were obtained from build UMD3.1 of the *B. taurus* genome sequence on the Ensembl database (http://www.ensembl.org)

*Minor allele frequencies (*MAF*) below 0.05 and **deviations from HWE ($p<0.05$) were not included in further statistical analysis

domain of the GHR protein (Blott et al. 2003). OPN_8514C-T is a substitution of a C for a T in intron 4 of the SPP1 gene. Looking at each genotype effect for those SNPs, the genotype T/A in GHR_140 showed a positive effect in reducing the SCS, while genotype T/T in OPN_8514C-T had a negative impact, increasing the SCS value.

For genes containing more than one SNP, combined genotypes were analyzed (Table 3). All existing combined genotypes were included in the association analysis. From all the possibilities of each combined genotype, only the LTF (lactoferrin gene) was shown to have all possible combinations (9/9). The other genotypes counts were CSN3 (casein kappa) (6/9), PRL (prolactin) (8/9), PPARGC1A (peroxisome proliferator-activated receptor-γ coactivator 1α) (10/27), OPN (6/9), and GH (growth hormone) (8/9). From the association study of the combined genotypes, only those corresponding to the OPN gene were found to be significantly associated with the SCS. The genotype AC/TT confirmed the results obtained during the analysis of the individual SNP effect, showing an increase in the SCS value (0.30 ± 0.13).

Heritabilities and other estimated variance components are included in Table 4, along with the estimated proportion of phenotypic variance accounted for the different random effects and its estimated standard error. The estimated heritability values ranged from 0.094 (no marker model) to 0.091. The proportions of additive genotypic variance explained by the

Table 2 Effect sizes per genotype class (and corresponding standard deviations) for SNPs and combined genotypes significantly associated, after false discovery rate (FDR) correction, with the somatic cell score (SCS)

SNP	Genotypes/maker effects (SD)					*p*-Value	Intercept
GHR_140	TT	TA	AA			0.008	4.25 (0.40)
	0	−0.37 (0.18)	−0.15 (0.07)				
OPN_8514C-T	CC	CT	TT			0.024	4.22 (0.41)
	0.00	0.17 (0.09)	0.38 (0.18)				
OPN	AA/TC	AA/TT	AC/TC	AC/TT	CC/TT	0.018	4.29 (0.46)
	0.019 (0.10)	0.19 (0.24)	0.05 (0.09)	0.30 (0.13)	0.04 (0.14)		

Table 3 Frequencies of combined genotypes

Locus	Combined genotype	Number	Frequency
LTF	AA/CC	7	0.01
	AA/CG	124	0.14
	AA/GG	415	0.47
	AC/CC	20	0.02
	AC/CG	149	0.17
	AC/GG	134	0.15
	CC/CC	12	0.01
	CC/CG	18	0.02
	CC/GG	6	0.01
CSN3	AA/CC	517	0.58
	AA/TT	12	0.01
	AA/CT	212	0.24
	AG/CC	132	0.15
	AG/CT	13	0.01
	GG/CC	5	0.01
PRL	CC/AA	7	0.01
	CC/AG	34	0.04
	CC/GG	31	0.03
	CT/AA	5	0.01
	CT/AG	132	0.14
	CT/GG	273	0.29
	TT/AG	28	0.03
	TT/GG	417	0.45
PPARGC1A	AA/GG/CC	37	0.04
	AG/GA/CC	58	0.06
	AG/GG/CC	218	0.24
	AG/GG/CT	29	0.03
	GG/AA/CC	14	0.02
	GG/GA/CC	140	0.15
	GG/GA/CT	26	0.03
	GG/GG/CC	298	0.33
	GG/GG/CT	83	0.09
	GG/GG/TT	3	0.00
OPN	AA/CC	232	0.28
	AA/TC	169	0.20
	AA/TT	19	0.02
	AC/TC	247	0.30
	AC/TT	91	0.11
	CC/TT	71	0.09
GH	CC/AA	131	0.14
	CC/AC	90	0.10
	CC/CC	24	0.03
	TC/AA	352	0.38
	TC/AC	112	0.12
	TC/CC	5	0.01
	TT/AA	199	0.22
	TT/AC	2	0.00

different markers were 0.025, 0.015, and 0.035 for GHR_140, OPN_8514C-T, and the combined OPN markers, respectively. An estimated repeatability value for the SCS in this dataset was 0.47.

Discussion

The heritability and repeatability values estimated in this study are consistent with previously published studies (Mrode and Swanson 1996; Evans and Berry 2005; Rupp and Boichard 1999). Markers found to be associated with the SCS in this study explained only a small proportion of the total genetic variance. This is due to the low heritability and the polygenic nature of the SCS, which is affected by a large number of variants throughout the genome.

In the present study, a panel of SNPs selected from different candidate genes or regions was evaluated. Several SNPs were discarded because of failure of the genotyping system (8), because they were not related to the aim of the study (3), or because of genetic constraints for the analysis (8).

Associations were detected for two individual SNPs and for combined genotypes of one of the candidate genes. The detected effects, even small effects, lie on two different genes, one directly related with the immune system (OPN) and the other that can be considered related with many biological functions.

Osteopontin (OPN) is a phosphorylated glycoprotein that plays a role in various biological processes. It is a cytokine produced by macrophages and activated T cells (Patarca et al. 1989), considered as an important regulator of the inflammatory response encoded by the gene SPP1 (secreted phosphoprotein 1). Among other properties found for osteopontin, it has been shown to confer resistance to several intracellular pathogens infections, through the recruitment and activation of macrophages (Weber et al. 2002). An analysis of the OPN gene by Alain et al. (2009) revealed that the OPN transcript was detected as being early expressed during mastitis in mammary somatic cells. In this study, we showed that different genotypes of the OPN_8514C-T marker significantly affected the SCS. Animals with the T/T genotype showed higher SCS values (0.38 ± 0.18, $p < 0.05$) than animals with other genotypes, a finding that was confirmed later by analysis of the combined genotypes of the polymorphisms in the OPN gene. Individuals with the combined genotype AC/TT were those who had the highest SCS (0.30 ± 0.13; $p<0.05$). The genotype C/T in OPN8514C-T was the most frequently found (half of our population), and the remaining animals had genotypes C/C and T/T at similar proportions (0.28 and 0.22, respectively). Previous studies have shown a significant association of OPN variants with milk composition traits like fat and protein percentage (Cohen et al. 2004; Schnabel et al. 2005), revealing similar genotypic frequencies in pure Holstein and Polish Holstein–Friesian herds to those found in our study (Leonard

Table 4 Summary of estimated variance components (and corresponding standard errors) for the different markers

	No marker	GHR_140	OPN_8514C-T	OPN
σ_e^2	1.012 (0.320)	1.010 (0.310)	1.011 (0.312)	1.007 (0.324)
σ_a^2	0.198 (0.053)	0.193 (0.049)	0.195 (0.051)	0.191 (0.050)
σ_{pe}^2	0.904 (0.027)	0.905 (0.025)	0.904 (0.031)	0.905 (0.050)
σ_p^2	2.114 (0.592)	2.107 (0.585)	2.110 (0.572)	2.105 (0.610)
h^2	0.094	0.092	0.092	0.091
$\%V_a SNP$[a]	–	0.025	0.015	0.035
$Vrat_e$[b]	0.479 (0.095)	0.478 (0.081)	0.478 (0.076)	0.476 (0.072)
$Vrat_{pe}$[c]	0.428 (0.087)	0.428 (0.083)	0.428 (0.079)	0.428 (0.076)

[a] Proportion of additive genotypic variance explained by SNPs

[b, c] Proportion of phenotypic variation accounted by random effects

et al. 2005; Khatib et al. 2007). In the former studies, the heterozygote C/T was the most abundant genotype in the populations. The C/T polymorphism at position 8514 of the OPN gene was previously associated with milk protein and fat percentages in 1,362 bulls obtained from 28 sire families (Leonard et al. 2005); however, to the best of our knowledge, this is the first time that an association between this polymorphism and the SCS is reported. The effect determined for the combined genotype was higher than the effect of the SNP OPN_8514C-T itself. It is well known that the power and accuracy of association mapping can be improved by grouping SNPs into haplotype blocks (Zhao et al. 2003). Many studies have been reported where the combined genotype or haplotype analysis resulted in the detection of a significant association with the trait, while single SNP analysis did not result in any significant association or presented higher *p*-values (Clark 2004; Lambert et al. 2013). Although this SNP has been studied in our population and should be validated for different populations, it has potential value as a molecular marker in a marker-assisted selection program.

The transmembrane receptor growth hormone encoded by the GHR gene, located on chromosome 20 (Frank et al. 1994), is a transducer of hormone action and plays a key role in lipid and carbohydrate metabolism (Lucy et al. 2001). It is expressed on numerous immune cells, including T cells, B cells, and monocytes, and is known to promote cell proliferation and/or survival (Taub et al. 2010). Several studies reported repeated mapping of QTLs for the SCS on chromosome 20, close to the GHR gene (Rodriguez-Zas et al. 2002; Ashwell et al. 2004; Ron et al. 2004; Casas and Snowder 2008).

The GHR_140 maker was originally described by Blott et al. (2003) as a substitution of an A for a T in exon 8 of the bovine growth hormone receptor gene. This change leads to a substitution of a phenylalanine for a tyrosine residue at position 279 (F279Y) located within the transmembrane domain of the GHR protein. In the association analysis of the GHR_140 (F279Y) marker, we have found that animals carrying the T/A allele showed significantly lower SCS values (-0.37 ± 0.18; $p<0.05$) than animals carrying other alleles. The minor allele represented in our population was 279Y, showing a frequency of 0.16; the observed genotypic frequencies found were 0.70 for the homozygous T/T, 0.26 for the heterozygous T/A, and 0.03 for the homozygous A/A. The low frequency of the homozygote YY in our study was consistent with other Holstein–Friesian populations from the Netherlands, New Zealand (Blott et al. 2003), the UK (Banos et al. 2008), and Italy (Fontanesi et al. 2009). Rahmatalla et al. (2011) found that the F279Y genotypes showed highly significant effects on milk and lactose yields and a significant effect on lowering the SCC for the 279Y allele. However, since the YY genotype occurs rarely in different Holstein populations, further analysis with larger populations should be conducted in order to verify the true effect of the genotype.

Polymorphisms in this gene have been found to be significantly associated with the SCS and production traits in different Holstein cows from several countries. Blott et al. (2003) mapped a QTL with a major effect on milk yield and composition, finding that this polymorphism was associated with this effect in Holstein and Jersey populations from different countries, pointing out that the SNP is the responsible nucleotide (quantitative trait nucleotide, QTN) or is tightly associated with the causal mutation. Viitala et al. (2006) confirmed that the F279Y SNP explained most of the QTL variance for milk and protein yield in Finnish Ayrshire dairy cattle, but they also found another association between the SNP PRLR_S18N (from a serine by asparagine residue change at position 18) with protein and fat yield. In another study, Waters et al. (2011) reported 32 new SNPs by resequencing of the GHR gene and also confirmed that the F279Y SNP is strongly associated with milk yield and fat/protein content. One of the 32 polymorphisms, GHR_19.1, was located in an intron 2,349 bp upstream of exon 3 and was associated with the SCS. Therefore, based on the mentioned evidence, this marker could be useful in a selection program to improve resistance to mastitis, together with other milk production traits. Although the 279Y allele is strongly associated with high milk yield and lower SCS, it occurs in low frequency in dairy cattle. Further studies should be conducted to clarify whether this polymorphism is also affecting other milk, reproductive, or/and fitness traits, which has led to a negative selection in dairy cows.

In conclusion, we reported here the association of two markers, one of which is not directly related with the immune functions, with the SCS in Argentinean dairy cattle. Based on these results, and in accordance with previous studies, it appears that not only the genes involved in the immune response and its basic functions, but also other genomic regions not directly related with the immune system are involved in resistance/susceptibility to mastitis. The OPN_8514C-T and GHR_140 genotypes and the OPN combined genotypes significantly affected the SCS in this herd, providing useful information for its application in breeding programs.

Acknowledgments This work was supported by Agencia Nacional de Promoción Científica y Tecnológica (PAE 37143, PICT 01523), INTA PNLEC-071051, and INTA AEBIO-245711.

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