https://doi.org/10.17221/43/2025-CJAS

Effects of *BRCA1* and *TLR1* SNPs on milk production performance and somatic cell score in Holstein, Simmental and crossbred Holstein cattle

Maja Maurić Maljković[1]*, *Tomislav Mašek*[2], *Marija Špehar*[3], *Kristina Starčević*[4]

[1]*Department of Animal Breeding and Livestock Production, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*
[2]*Department of Animal Nutrition and Dietetics, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*
[3]*Croatian Agency for Agriculture and Food, Zagreb, Croatia*
[4]*Department of Chemistry and Biochemistry, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

**Corresponding author: mmauric@vef.unizg.hr*

Citation: Maurić Maljković M., Mašek T., Špehar M., Starčević K. (2025): Effects of *BRCA1* and *TLR1* SNPs on milk production performance and somatic cell score in Holstein, Simmental and crossbred Holstein cattle. Czech J. Anim. Sci, 70: 267–275.

Abstract: Mastitis is a complex, common and economically problematic issue in dairy cattle production. In this study, breast cancer 1 (*BRCA1*) and toll-like receptor 1 (*TLR1*) genes were taken as candidate genes for mastitis resistance. This study investigated whether *BRCA1* and *TLR1* genes were associated with milk production traits (daily milk yield, fat, and protein content) and somatic cell score (SCS). A total of 105 cows (25 Holstein, 48 Simmental, and 32 crossbred Holstein) were genotyped using the PCR-RFLP method. Cows with the *BRCA1* c.46126G>T *GG* genotype had significantly lower SCS than the other genotypes. The *TLR1* g.60438363C>T SNP influenced the protein content in all cows, with genotype *CC* having a higher content than *TC*. The same SNP in the Simmental breed showed that cows with the *CC* genotype had significantly higher SCS than the heterozygote. Cows with the *GG* genotype of *TLR1* g.60437324A>G had significantly lower SCS and higher fat and protein content than the heterozygote. The results of this study indicate that *BRCA1* c.46126G>T and *TLR1* g.60437324A>G SNPs could be useful for improving mastitis resistance in dairy cattle through marker-assisted selection.

Keywords: cow; mastitis; mastitis resistance; somatic cell count; udder health

Mastitis, an inflammatory mammary gland disease, is a prevalent and economically significant issue in dairy cattle production. It causes reduced milk yield, increased veterinary costs, potential culling of affected animals, and it severely impacts animal welfare (Zhang et al. 2009; Yuan et al. 2012; Bai et al. 2022; Yang et al. 2022). It is caused by various pathogens, including bacteria, viruses, yeasts, and mycoplasma (Russell et al. 2012; Magotra et al. 2020; Bai et al. 2022). However, genetics, management practices, and cattle health also play important roles (Zhang et al. 2009; Yuan et al. 2012). The

https://doi.org/10.17221/43/2025-CJAS

differences in mastitis tolerance between breeds indicate the presence of genetic variations (Asaf et al. 2015). As genetic improvement is influenced through long-term selection, recent research has increasingly focused on the genetic factors contributing to mastitis susceptibility, such as the gene variation that controls the immune response (Russell et al. 2012; Yuan et al. 2012; Bjelka and Novak 2020). In this context, our study focused on specific genetic variants (SNPs) within candidate genes previously implicated in mastitis resistance and immune function, aiming to further analyse their association with udder health and milk production traits.

The breast cancer 1 (*BRCA1*) gene, a RING-type chain finger (RNF) family member, belongs to a class of tumour suppressor genes. It works in the process of DNA damage repair, cell cycle regulation, transcriptional regulation, and other important pathways that maintain genome stability (Krum et al. 2003; Yuan et al. 2012; Asaf et al. 2015; Biendima et al. 2017; Magotra et al. 2020; Daldaban et al. 2021). *BRCA1* is widely recognised for its association with breast cancer in humans (Krum et al. 2003; Wang et al. 2012; Yuan et al. 2012). Since variations in *BRCA1* expression may affect the mammary gland ability to respond to infections, it has emerged as a potential candidate gene in the context of mastitis in dairy cattle, although there have not been done many studies yet (Yuan et al. 2012; Asaf et al. 2015; Biendima et al. 2017; Magotra et al. 2020; Daldaban et al. 2021). In cattle, it has been mapped to chromosome 19 (BTA19) (Krum et al. 2003), within or near the genomic region of a somatic cell score (SCS) QTL (Daetwyler et al. 2008; Yuan et al. 2012). A non-synonymous SNP in exon 13, at position 46126 of the bovine *BRCA1* gene (NC_007317.4:c.46126 G>T), that is causing a change from tyrosine (allele *T*) to aspartic acid (allele *G*), has been proposed as a potential marker for SCS (Yuan et al. 2012).

Toll-like receptors (TLRs) are a structurally conserved type I membrane-bound class of pattern recognition receptors (PRRs). They play an important role in the innate immune response, as they can differentiate within a wide range of permanent microorganism structures called pathogen-associated molecular patterns (PAMPs) (Russell et al. 2012; Bhaladhare et al. 2019; Sameer and Nissar 2021; Yang et al. 2022). Each TLR recognises specific PAMPs (Russell et al. 2012; Bartens et al. 2021; Sameer and Nissar 2021). TLR1, generally in association with TLR2 as a TLR1/TLR2 heterodimer, may be activated by a wide variety of bacterial PAMPs, such as lipoproteins and lipopolysaccharides from the Gram-positive and Gram-negative bacterial cell wall (Mucha et al. 2009; Russell et al. 2012; Arslan et al. 2018). Two SNPs located within bovine *TLR1* exon 5, a synonymous *TLR1* g.798C>T SNP (NC_007304.4:g.60438363C>T) and a non-synonymous g.1762A>G mutation (NC_007304.4:g.60437324A>G) that causes a change from isoleucine (allele *G*) to valine (allele *A*), were potentially linked to clinical mastitis and SCS (Li et al. 2009; Russell et al. 2012). The SNP offsets are given relative to their position (bp) from the A nucleotide of the ATG codon (Russell et al. 2012).

Clinical mastitis (CM) can serve as a highly accurate indicator connecting mastitis to genetic factors. However, its incidence data are costly and challenging to collect (Russell et al. 2012; Asaf et al. 2014) and overlook the most prevalent form, subclinical mastitis (Roldan-Montes et al. 2020). On the other hand, somatic cell count (SCC) data are readily available and, as previous studies have indicated, represent the most appropriate single trait for reducing the incidence of mastitis through indirect selection (Yuan et al. 2012; Asaf et al. 2014).

Identifying SNPs within genes involved in maintaining the genome stability or the mammary innate immune response, such as *BRCA1* and *TLR1*, is receiving particular interest like DNA markers for lower SCC or SCS (Russell et al. 2012; Yuan et al. 2012). Therefore, the present study aims to investigate the relationship between the *BRCA1* and *TLR1* SNPs and milk production traits – daily milk yield, fat and protein content, and SCS.

MATERIAL AND METHODS

Animals

One hundred and five milk samples were collected from 25 Holstein, 48 Simmental, and 32 crossbred Holstein (Holstein × Simmental) clinically healthy cows. Samples were provided by one farm located in Zagreb County, Croatia. Information about milk production traits (daily milk yield – DMY, fat content – FC, protein content – PC, and SCS) for the first lactating cows was provided

https://doi.org/10.17221/43/2025-CJAS

by the Croatian Agency for Agriculture and Food, responsible for milk recording systems according to the International Committee for Animal Recording rules (ICAR 2011). All cows were fed a basal total mixed ration (TMR) diet consisting of haylage and maize concentrate and had constant access to drinking water. They were milked twice a day. One morning milk sample per cow was collected for DNA isolation.

Genotyping

DNA was isolated from milk samples (350 µl) using the PathoProof™ DNA Extraction Kit (Thermo Fisher Scientific, Vantaa, Finland), following the protocol instructions. The primers, PCR product length, PCR conditions, restriction enzymes, and restriction fragments are given in Table 1. The PCR products (5 µl) were digested with the corresponding restriction enzymes (Table 1): *Hpy*188I (New England Biolabs Inc, Hitchin, UK), *Mbo*II (Takara Bio Inc., Kusatsu, Japan), and *Fba*I (Takara Bio Inc., Kusatsu, Japan) following the supplier's manual. The resulting fragments were separated on a 3% agarose gel.

Statistical analysis

Calculation of allele and genotype frequencies, polymorphism deviation from the Hardy-Weinberg equilibrium and population genetic indices (observed heterozygosity – H_O, expected heterozygosity – H_E, and fixation index – F_{IS}) were performed using POPGENE32 software v1.32 (Yeh et al. 2000). All traits were analysed by the least squares method using the general linear model procedure in the SAS statistical package (SAS software, v9.3; SAS Institute Inc. Cary, NC, USA). The following model was used:

$$y_{ijklm} = \mu + B_i + G_{ij} + C_k + b_1\left(x_{ijkl} - \bar{x}\right)^2 + \\ + b_2\left(t_{ijklm} - \bar{t}_{ijklm}\right)^2 + e_{ijklm} \quad (1)$$

where:

y_{ijklm} – observed trait (DMY, FC, PC, and SCS);
µ – overall mean;
B_i, G_j, C_k – breed (B_i), gene (G_j), and calving season (C_k) = fixed class effects;
b_1, b_2 – regression coefficients
e_{ijkl} – residual error.

Table 1. The primers, PCR product length, PCR conditions, restriction enzymes and restriction fragments for the studied SNPs

SNP name	Primers (5'→3')	PCR product length (bp)	PCR condition	Restriction enzyme	Restriction fragments (allele, bp)	Literature
BRCA1 c.46126G>T	F: GTGTGATTAGTCCTTTCACAAGC R: TCTCCACCAGAGCAGATGAAAT	272	94 °C/5 min → 35 × (94 °C/30 s, 58.8 °C/30 s, 72°C/30 s) → 72 °C/8 min	*Hpy*188I	*G*: 272 *T*: 184, 88	Yuan et al. (2012)
TLR1 g.60438363C>T	F: GCACCACAGTGAGTCTGGAA R: GTACGCCAAACCAACTGGAG	181	95 °C/3 min → 34 × (95 °C/30 s, 59 °C/30 s, 72 °C/30 s) → 72 °C/10 min	*Mbo*II	*T*: 181 *C*: 142, 39	Russell et al. (2012)
TLR1 g.60437324A>G	F: AGGGCTGGCCTGAGTCTTAT R: TTCTTCACCCAGGCAGAATC	316	95 °C/3 min → 34 × (95 °C/30 s, 60 °C/30 s, 72 °C/30 s) → 72 °C/10 min	*Fba*I	*G*: 316 *A*: 208, 108	Russell et al. (2012)

PCR = polymerase chain reaction; SNP = single nucleotide polymorphism

https://doi.org/10.17221/43/2025-CJAS

Age at first calving (x_{ijkl}) and days in milk (t_{ijklm}) were fitted in the model as quadratic regression. Before the analysis, a logarithmic transformation for SCC was performed to obtain a normal distribution (Ali and Shook 1980).

RESULTS

Frequencies, Hardy-Weinberg equilibrium, and genetic indices

The allele and genotype frequencies, Hardy-Weinberg equilibrium, and genetic indices of the studied SNPs are presented in Table 2. In all three groups, regarding the *BRCA1* gene, the *GT* genotype prevailed over both homozygous genotypes, with allele *G* being the more frequent (Table 2). In *TLR1* g.60438363C>T, the heterozygous *TC* was the most frequent among all cows, with allele *T* being more prevalent in the Holstein and Simmental breeds, while allele *C* was more common in crossbreds (Table 2). Most animals included in the study were genotyped as *GG* homozygous genotypes for the *TLR1* g.60437324A>G SNP. Allele *A* was slightly more frequent in both breeds, while allele *G* occurred more frequently in the crossbreds (Table 2). The distribution of genotypes did not fol-

Table 2. Allelic and genotypic frequencies, Hardy-Weinberg equilibrium, and genetic indices of *BRCA1* c.46126G>T, *TLR1* g.60438363C>T and g.60437324A>G in Simmental, Holstein and crossbred Holstein cattle (n = 105)

Gene	Breed	Allele	Allele frequency	Genotype	n	Genotype frequency	χ^2	P-value	H_O	H_E	F_{IS}
BRCA1 c.46126G>T	Holstein	*G*	0.560	*GG*	8	0.320	0.05	0.82	0.480	0.503	0.026
		T	0.440	*GT*	12	0.480					
				TT	5	0.200					
	Simmental	*G*	0.583	*GG*	14	0.292	1.73	0.19	0.583	0.491	–0.200
		T	0.417	*GT*	28	0.583					
				TT	6	0.125					
	crossbred Holstein	*G*	0.625	*GG*	9	0.281	7.46	0.01	0.688	0.476	–0.467
		T	0.375	*GT*	22	0.688					
				TT	1	0.031					
TLR1 g.60438363C>T	Holstein	*C*	0.440	*CC*	6	0.240	1.09	0.30	0.400	0.503	0.188
		T	0.560	*TC*	10	0.400					
				TT	9	0.360					
	Simmental	*C*	0.375	*CC*	6	0.125	0.15	0.70	0.500	0.474	–0.067
		T	0.625	*TC*	24	0.500					
				TT	18	0.375					
	crossbred Holstein	*C*	0.609	*CC*	10	0.313	1.72	0.19	0.594	0.484	–0.247
		T	0.391	*TC*	19	0.594					
				TT	3	0.094					
TLR1 g.60437324A>G	Holstein	*A*	0.580	*AA*	9	0.360	0.34	0.56	0.440	0.497	0.097
		G	0.420	*GA*	11	0.440					
				GG	5	0.200					
	Simmental	*A*	0.510	*AA*	10	0.208	1.89	0.17	0.604	0.505	–0.209
		G	0.490	*GA*	29	0.604					
				GG	9	0.188					
	crossbred Holstein	*A*	0.453	*AA*	6	0.188	0.10	0.75	0.531	0.504	–0.072
		G	0.547	*GA*	17	0.531					
				GG	9	0.281					

F_{IS} = fixation index; H_E = expected heterozygosity; H_O = observed heterozygosity

https://doi.org/10.17221/43/2025-CJAS

Table 3. Effect of *BRCA1* c.46126G>T, *TLR1* g.60438363C>T and g.60437324A>G gene on daily milk yield (DMY), fat content (FC), protein content (PC) and somatic cell score (SCS)

Gene SNP	Genotype	DMY (kg) (LSM ± SE)	FC (%) (LSM ± SE)	PC (%) (LSM ± SE)	SCS (LSM ± SE)
BRCA1 c.46126G>T	*GG*	20.70 ± 0.91	4.10 ± 0.16	3.59 ± 0.07	8.21 ± 0.35^{ab}
	GT	19.97 ± 0.73	4.27 ± 0.13	3.75 ± 0.06	9.24 ± 0.28^{a}
	TT	21.22 ± 1.71	4.15 ± 0.30	3.52 ± 0.13	9.66 ± 0.65^{b}
TLR1 g.60438363C>T	*CC*	21,.5 ± 0.98	4.34 ± 0.18	3.83 ± 0.08^{a}	8.95 ± 0.40
	TC	20.35 ± 0.67	4.22 ± 0.12	3.63 ± 0.07^{a}	8.89 ± 0.27
	TT	19.80 ± 1.06	4.36 ± 0.21	3.63 ± 0.08	8.88 ± 0.50
TLR1 g.60437324A>G	*AA*	20.17 ± 1.10	4.33 ± 0.19	3.61 ± 0.09^{a}	8.97 ± 0.47
	GA	20.70 ± 0.69	4.06 ± 0.12^{a}	3.62 ± 0.06^{b}	9.29 ± 0.28^{a}
	GG	19.87 ± 1.08	4.61 ± 0.19^{a}	3.88 ± 0.09^{a,b}	8.22 ± 0.43^{a}

Values are expressed as least squares mean (LSM) ±standard error (SE)

^{a,b}Values with the same superscripts within the same column in the same locus denote significant differences ($P < 0.05$)

Table 4. Effect of *BRCA1* c.46126G>T, *TLR1* g.60438363C>T and g.60437324A>G gene on daily milk yield (DMY), fat content (FC), protein content (PC) and somatic cell score (SCS) in Simmental (S), Holstein (H) and crossbred Holstein (H × S) cattle

Breed	Trait (LSM ± SE)	*BRCA1* c.46126G>T			*TLR1* g.60438363C>T			*TLR1* g.60437324A>G		
		GG	*GT*	*TT*	*CC*	*TC*	*TT*	*AA*	*GA*	*GG*
H	DMY (kg)	21.85 ± 1.77	21.03 ± 1.39	21.01 ± 2.07	22.19 ± 1.72	22.42 ± 1.50	19.66 ± 1.58	20.26 ± 1.63	23.77 ± 1.56	20.17 ± 1.99
	FC (%)	4.19 ± 0.31	4.56 ± 0.26	4.21 ± 0.37	4.52 ± 0.29	4.68 ± 0.26	3.95 ± 0.29	4.33 ± 0.27	4.34 ± 0.27	4.95 ± 0.37
	PC (%)	3.43 ± 0.14	3.71 ± 0.11^{a}	3.31 ± 0.16^{a}	3.64 ± 0.14	3.61 ± 0.13	3.49 ± 0.13	3.47 ± 0.13^{a}	3.46 ± 0.13^{b}	3.94 ± 0.16^{ab}
	SCS	8.85 ± 0.67	9.82 ± 0.54	10.22 ± 0.81	8.98 ± 0.70	10.15 ± 0.63	9.78 ± 0.64	9.08 ± 0.65	10.17 ± 0.65	9.13 ± 0.80
S	DMY (kg)	19.54 ± 1.32	18.72 ± 0.95	16.44 ± 1.89	19.84 ± 1.60	19.19 ± 0.91	17.37 ± 1.30	18.19 ± 2.12	18.68 ± 0.90	18.89 ± 1.47
	FC (%)	3.92 ± 0.23	3.93 ± 0.17	4.32 ± 0.33	4.08 ± 0.28	3.98 ± 0.16	4.06 ± 0.22	4.30 ± 0.35	3.93 ± 0.15	4.27 ± 0.25
	PC (%)	3.48 ± 0.10^{a}	3.68 ± 0.07	3.85 ± 0.14^{a}	3.78 ± 0.14	3.58 ± 0.08	3.70 ± 0.11	3.44 ± 0.17	3.63 ± 0.07	3.79 ± 0.12
	SCS	8.15 ± 0.50^{a}	8,83 ± 0.36^{b}	10.64 ± 0.72^{ab}	8.21 ± 0.65	8.82 ± 0.37	9.22 ± 0.53	8.52 ± 0.85	8.95 ± 0.36	8.24 ± 0.59
H × S	DMY (kg)	20.69 ± 1.57	20.18 ± 1.13	26.22 ± 4.20	22.31 ± 1.68	19.44 ± 1.16	22.36 ± 2.56	22,05 ± 1,85	19.65 ± 1.32	20.55 ± 1.60
	FC (%)	3.92 ± 0.75	4.31 ± 0.21	4.10 ± 0.30	4.41 ± 0.29	4.01 ± 0.21	5.07 ± 0.54	4,38 ± 0,34	3.93 ± 0.23	4.60 ± 0.27
	PC (%)	3.42 ± 0.32	3.88 ± 0.09	3.42 ± 0.32	4.06 ± 0.14^{a}	3.68 ± 0.10^{a}	3.71 ± 0.26	3,90 ± 0,17	3.77 ± 0.11	3.90 ± 0.13
	SCS	8.13 ± 1.60	9.08 ± 0.45	8.13 ± 1.60	9.64 ± 0.68^{a}	7.71 ± 0.48^{a}	7.63 ± 1.27	9,32 ± 0,82^{a}	8.75 ± 0.53	7.29 ± 0.64^{a}

Values are expressed as least squares mean (LSM) ± standard error (SE)

^{a,b}Values with the same superscripts within the same column in the same locus denote significant differences ($P < 0.05$)

https://doi.org/10.17221/43/2025-CJAS

low the Hardy-Weinberg equilibrium only in crossbred Holstein for the *BRCA1* gene, which was accompanied by higher H_O than H_E (0.688 and 0.476, respectively) and, accordingly, a high negative F_{IS} (–0.467). A heterozygosity deficit was identified only in the Holstein breed for all three SNPs, being small for *BRCA1* c.46126G>T and *TLR1* g.60437324A>G, and somewhat higher for *TLR1* g.60438363C>T (0.026, 0.097, and 0.188, respectively).

Associations between single SNPs and milk production traits and SCS

The effects of the SNPs on milk production traits and SCS were analysed and they are presented for all cows together in Table 3 and separately by breed in Table 4.

The effect of *BRCA1* c.46126G>T genotypes on the studied traits showed that the *GG* genotype had a significantly lower SCS than the *GT* and *TT* genotypes ($P < 0.05$), while it had no effects on other traits (Table 3). When analysed by breed, SCS was significantly higher in cows with the *TT* genotype than in the others ($P < 0.05$). Also, it was found that *BRCA1* c.46126G>T influenced the protein content in Holstein and Simmental cows, with the *GT* genotype having a higher content than *TT* in Holstein cows and the *TT* genotype having a higher content than *GG* in the Simmental ($P < 0.05$; Table 4).

The *TLR1* g.60438363C>T SNP influenced the PC, with genotype *CC* having a higher content than *TC* in all cows and Simmental separately ($P < 0.05$; Tables 3 and 4). Also, Simmental cows with the *CC* genotype had significantly higher SCS than those with the *TC* genotype ($P < 0.05$).

In all cows, the *GG* genotype of *TLR1* g.60437324A>G had significantly lower SCS and higher FC and PC than the heterozygote (and *AA* homozygote for protein content; $P < 0.05$; Table 3). The same genotype, *GG*, also showed higher PC than the other two in Holstein cows and lower SCS than *AA* in crossbred cows ($P < 0.05$; Table 4).

DISCUSSION

Although there is research on *BRCA* and *TLR* genes, there are few reports on the polymorphisms studied here, in both purebred and crossbred cattle. Therefore, this study investigated the frequencies of one *BRCA1* and two *TLR1* SNPs and their effects on milk production traits and SCS in purebred Holstein and Simmental cows and crossbred Holstein × Simmental cows.

BRCA1 c.46126G>T

In the present study, we investigated the relationship between milk production traits and SCS and one SNP in the *BRCA1* gene, which has been reported as a potential candidate gene for detecting mastitis in Holstein, Sanhe, Simmental (Yuan et al. 2012), and Karan Fries cattle (*Bos taurus* × *Bos indicus*; Magotra et al. 2020). This non-synonymous SNP causes a change from tyrosine (allele *T*) to aspartic acid (allele *G*) at position 46126 of the bovine *BRCA1* gene.

The results from the present study align with Yuan et al. (2012), showing *G* as the predominant allele in all researched breeds (0.644 9–0.718 7). Contrarily, Magotra et al. (2020) found allele *T* to be more frequent (0.578). The genotype frequencies varied among the three studies: Yuan et al. (2012) reported that genotype *GG* was the most common in all three breeds (0.504 7–0.593 8), while Magotra et al. (2020) identified *TT* (0.412). In contrast, we found genotype *GT* to be the most common in purebred and crossbred cows (Table 2). This discrepancy might reflect variations in breed, herd, and selection.

BRCA1 c.46126G>T is significantly associated with SCS in the analysed animals. Cows with the *GG* genotype had significantly lower values than those with the *GT* and *TT* genotypes ($P < 0.05$; Table 3) in all cows and in the Simmental breed separately (*TT* was significantly higher than *GT* and *GG*; $P < 0.05$; Table 4).

Our findings fully support the observation of previous studies that cows with the *GG* genotype of *BRCA1* c.46126G>T had significantly lower SCS (Yuan et al. 2012) and were less susceptible to mastitis (Magotra et al. 2020). Apart from SCS, when analysed separately by breed, this SNP influenced PC ($P < 0.05$; Table 4).

With the combined results from these studies in different herds and breeds, this SNP is indicative of a potential marker for different mastitis susceptibility, even though the exact mechanism and effects should be clarified further.

https://doi.org/10.17221/43/2025-CJAS

TLR1 g.60438363C>T

Other studies have shown that *TLR2* might be a good candidate gene for mastitis susceptibility (Opsal et al. 2006; Bartens et al. 2021). Since TLR2 is primarily present as a heterodimer that depends on its association with TLR1 or TLR6 for the proper function, the *TLR6-TLR1-TLR10* gene cluster could also be a potential candidate region (Opsal et al. 2006), and, therefore, two *TLR1* SNPs were investigated in this study.

In *TLR1* g.60438363C>T for the crossbred cows, the more common allele was *C* (0.609), which aligns with the findings of Opsal et al. (2006) – 0.68 and Russell et al. (2012) – 0.543. In contrast, the other two breeds had allele *T* as the more common one (0.56 in Holstein and 0.625 in Simmental), as noted by Seabury et al. (2007) – 0.60. Regarding the genotypic frequencies, the heterozygote was the most common in all three breeds from this study (Table 2) and in Russell et al. (2012) – 0.49. To our knowledge, *TLR1* g.60438363C>T was not previously analysed for association with milk production traits and SCS. In this study, the *CC* homozygote showed higher PC in all cows and the crossbreds separately, and higher SCS in the crossbreds. In the study of Bjelka and Novak (2020), *TLR1* g.60438363C>T was associated with maternal calving ease and production longevity. The *TLR1* g.60438363C>T is a synonymous SNP, thus not causing any structural or functional change in TLR1 response. Consequently, it might be that it is not the actual causative SNP but it is linked to other functional mutations, so this should be investigated further in a larger-scale study.

TLR1 g.60437324A>G

Allele *A* was more common in Holstein and Simmental, as reported by Seabury et al. (2007) – 0.70, while allele *G* was more frequent in the crossbreds, as noted by Russell et al. (2012) – 0.505. The prevalence of either allele in our study and those of Seabury et al. (2007) and Russell et al. (2012) was relatively low, with all frequencies slightly above 0.5. Consequently, all studied breeds, and the Holstein Friesian cows in Russell et al. (2012) – 0.46 had heterozygotes as the most common genotype.

The genotype *GG*, having higher FC (all cows) and PC (all cows and crossbreds), aligned with the findings of Russell et al. (2012), even though their differences were not statistically significant. The same authors reported that *AA* and *AG* animals had higher rates of clinical mastitis (0.69 and 0.66 cases/cow/year, respectively) than *GG* animals (0.38 cases/cow/year). However, these differences were not statistically significant. Also, they found no differences in mean SCC. In this study, all cows and, separately, crossbred Holstein cows with the *GG* genotype had significantly lower SCS, following the aforementioned clinical mastitis reports. Similarly, a study on Chinese Holsteins indicated that cows with the *GG* genotype had lower SCS and were classified as more resistant to mastitis (Li et al. 2009).

Even though the g.60437324A>G SNP is a non-synonymous mutation that causes a change from isoleucine (allele *G*) to valine (allele *A*), it remains unclear whether it causatively affects the variable TLR1 response. Both are non-polar branched amino acids with hydrophobic properties, so a substitution within the transmembrane domain might not impact the tertiary structure (Russell et al. 2012). However, since the same genotype (*GG*) is associated with lower SCS and higher FC and PC, and the *TLR6-TLR1-TLR10* gene cluster is located ~28 Mb from the casein gene cluster on BTA 6 (Threadgill and Womack 1990; Russell et al. 2012) and overlaps with a QTL for clinical mastitis (Klungland et al. 2001; Novak 2014), this indicates a functional association between FC, PC, and SCS/mastitis susceptibility. As dairy cattle are often bred for higher FC and PC, this might also influence the selection for some *TLR1* genotypes (Russell et al. 2012). Bjelka and Novak (2020) also associated this SNP with production longevity.

CONCLUSION

Results from the association analysis in this study indicate that the *GG BRCA1* c.46126G>T genotype and *GG TLR1* g.60437324A>G genotype have lower SCS/mastitis susceptibility, and that *BRCA1* and *TLR1* could be used as candidate genes or molecular markers in long-term breeding strategies aimed at improving mastitis resistance in dairy cattle. Mastitis is a complex and polygenic disease, and the relationship between genetic predisposition and environmental factors is essential in understanding the susceptibility of dairy cattle to mastitis. Nevertheless, selecting more resistant genotypes could enhance the overall genetic resistance and

https://doi.org/10.17221/43/2025-CJAS

reduce some of the infection pressure. However, future research should continue investigating these interactions and assess whether breeding for lower-risk combinations would not negatively impact milk production or health traits.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Ali AKA, Shook GE. An optimum transformation for somatic cell concentration in milk. J Dairy Sci. 1980 Mar; 63(3):487-90.

Arslan K, Akyuz B, Aksel EG, Ozdemir F, Cinar MU. Polymorphisms of TLR1, TLR4 and SLC11A1 genes in some cattle breeds reared in Turkey. J Agric Sci. 2018 Dec 5; 24(4):547-53.

Asaf VNM, Bhushan B, Panigrahi M, Kumar A, Dewangan P, Gaur GK, Sharma D. Lack of association of allelic variants of BRCA1 gene with mastitis susceptibility in Vrindavani cattle. Indian J Anim Sci. 2015 Jan;85(1):81-3.

Asaf VNM, Kumar A, Rahim A, Sebastian R, Mohan V, Dewangan P, Panigrahi M. An overview on single nucleotide polymorphism studies in mastitis research. Vet World. 2014 Jun;7(6):416-21.

Bai X, Wang X, Lin T, Dong W, Gao Y, Ji P, Zhang Y, Zhao X, Zhang Q. Toll-like receptor 2 is associated with the immune response, apoptosis, and angiogenesis in the mammary glands of dairy cows with clinical mastitis. Int J Mol Sci. 2022 Sep 14;23(18):10717.

Bartens MC, Gibson AJ, Etherington GJ, Di Palma F, Holder A, Werling D, Willcocks S. Single nucleotide polymorphisms in the bovine TLR2 extracellular domain contribute to breed and species-specific innate immune functionality. Front Immunol. 2021 Dec 23;12:764390.

Bhaladhare A, Yadav R, Baqir M, Chauhan A, Sonwane A, Kumar A, Singh RV, Kumar S, Kumar P, Kumar S, Bhushan B. Association study of an SNP in TLR1 gene with susceptibility to bovine tuberculosis in cows. J Entomol Zool Stud. 2019 Apr 13;7(3):248-52.

Biendima CC, Ramos SC, Uy MRD, Mingala CN. Molecular characterization of BRCA1 as candidate gene marker for subclinical mastitis in dairy water buffaloes (Bubalus bubalis). Philipp J Sci. 2017 Sep;146(83):293-8.

Bjelka M, Novak K. Association of TLR gene variants in a Czech Red Pied cattle population with reproductive traits. Vet Immunol Immunopathol. 2020 Feb;220:109997.

Daetwyler HD, Schenkel FS, Sargolzaei M, Robinson JAB. A genome scan to detect quantitative trait loci for economically important traits in Holstein cattle using two methods and a dense single nucleotide polymorphism map. J Dairy Sci. 2008 Aug;91(8):3225-36.

Daldaban F, Arslan K, Akcay A, Sohel MH, Akyuz B. Association of BRCA1 (G22231T, T25025A, C28300A) polymorphisms with subclinical mastitis and milk yields in Holstein cattle. Harran Univ Vet Fak Derg. 2021 Feb; 10(1):12-9.

ICAR – International Committee for Animal Recording. Guidelines approved by the General Assembly held in Riga, Latvia, on 31 May to 4 June 2010. Roma, Italia; 2011. p. 229-56.

Klungland H, Sabry A, Heringstad B, Olsen HG, Gomez-Raya L, Vage DI, Olsaker I, Odegard J, Klemetsdal G, Schulman N, Vilkki J, Ruane J, Aasland M, Ronningen K, Lien S. Quantitative trait loci affecting clinical mastitis and somatic cell count in dairy cattle. Mamm Genome. 2001 Nov;12(11):837-42.

Krum SA, Womack JE, Lane TF. Bovine BRCA1 shows classic responses to genotoxic stress but low in vitro transcriptional activation activity. Oncogene. 2003 Sep;22(38):6032-44.

Li C, Shi W, Chu M, An Y, Chen H, Di R, Fang L. Polymorphisms of TLR1 gene and their relationship with somatic cell score in Holstein cows. Sci Agric Sin. 2009;42(6):2118-25.

Magotra A, Gupta ID, Ahmad T, Alex R. Polymorphism in DNA repair gene BRCA1 associated with clinical mastitis and production traits in indigenous dairy cattle. Res Vet Sci. 2020 Dec;133:194-201.

Mucha R, Bhide MR, Chakurkar EB, Novak M, Mikula Sr I. Toll-like receptors TLR1, TLR2 and TLR4 gene mutations and natural resistance to Mycobacterium avium subsp. paratuberculosis infection in cattle. Vet Immunol Immunopathol. 2009 Apr 15;128(4):381-8.

Novak K. Functional polymorphisms in Toll-like receptor genes for innate immunity in farm animals. Vet Immunol Immunopathol. 2014 Jan 15;157(1-2):1-11.

Opsal MA, Vage DI, Hayes B, Berget I, Lien S. Genomic organisation and transcript profiling of the bovine toll-like receptor gene cluster TLR6-TLR1-TLR10. Gene. 2006 Dec 15;384:45-50.

Roldan-Montes V, Cardoso DF, Hurtado-Lugo NA, do Nascimento AV, Santos DJA, Scalez DCB, de Freitas AC, Herrera AC, Albuquerque LG, de Camargo GMF, Tonhati H. Polymorphisms in TLR4 gene associated with somatic cell score in water buffaloes (Bubalus bubalis). Front Vet Sci. 2020 Nov 5;7:568249.

Russell CD, Widdison S, Leigh JA, Coffey TJ. Identification of single nucleotide polymorphisms in the bovine Toll-

https://doi.org/10.17221/43/2025-CJAS

like receptor 1 gene and association with health traits in cattle. Vet Res. 2012 Mar 14:43:17.

Sameer AS, Nissar S. Toll-Like Receptors (TLRs): Structure, functions, signaling, and role of their polymorphisms in colorectal cancer susceptibility. Biomed Res Int. 2021 Sep 12;2021:1157023.

Seabury CM, Cargill EJ, Womack JE. Sequence variability and protein domain architectures for bovine Toll-like receptors 1, 5, and 10. Genomics. 2007 Oct;90(4):502-15.

Threadgill DW, Womack JE. Genomic analysis of the bovine milk protein genes. Nucleic Acids Res. 1990 Dec 11;18(23): 6935-42.

Wang F, Fang Q, Ge Z, Yu N, Xu S, Fan X. Common BRCA1 and BRCA2 mutations in breast cancer families: A meta-analysis from systematic review. Mol Biol Rep. 2012 Mar; 39(3):2109-18.

Yang J, Liu Y, Lin C, Yan R, Li Z, Chen Q, Zhang H, Xu H, Chen X, Chen Y, Guo A, Hu C. Regularity of toll-like receptors in bovine mammary epithelial cells induced by Mycoplasma bovis. Front Vet Sci. 2022 Apr 7;9:846700.

Yeh FC, Yang R, Boyle TJ, Ye Z, Xiyan JM. POPGENE 32, Microsoft Window-based Freeware for Population Genetic Analysis, Version 1.32. Canada: Molecular Biology and Biotechnology Centre, University of Alberta; 2000.

Yuan Z, Li J, Li J, Zhang L, Gao X, Gao HJ, Xu S. Investigation on BRCA1 SNPs and its effects on mastitis in Chinese commercial cattle. Gene. 2012 Aug 15;505(1):190-4.

Zhang LP, Gan QF, Ma TH, Li HD, Wang XP, Li JY, Gao X, Chen JB, Ren HY, Xu SZ. Toll-like receptor 2 gene polymorphism and its relationship with SCS in dairy cattle. Anim Biotechnol. 2009 Feb;20(3):87-95.

Received: April 2, 2025
Accepted: July 8, 2025
Published online: July 29, 2025