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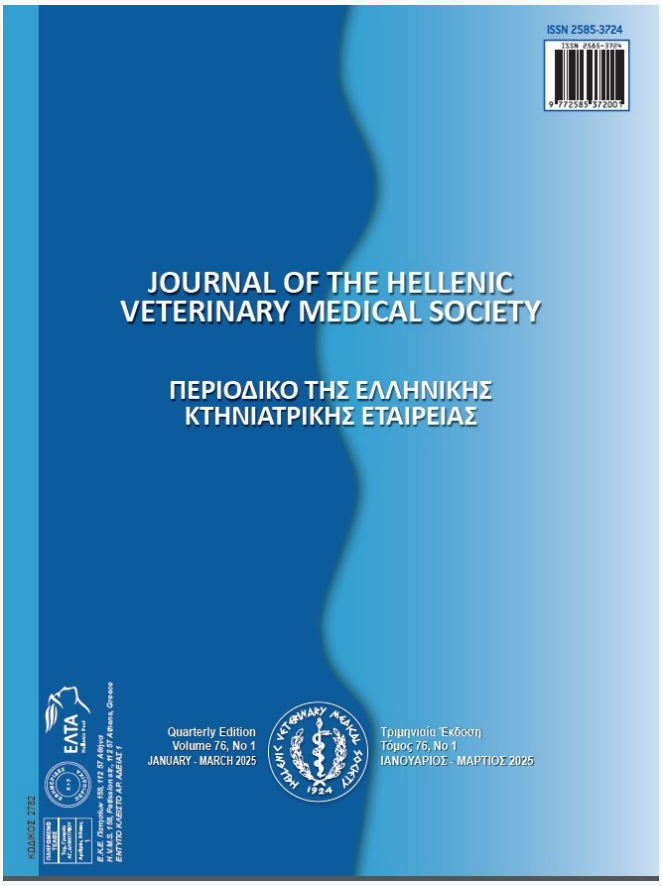


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*J Metin Kiyici, F Daldaban, K Arslan, B Akyüz, M Kaliber, MU Çinar*

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# Effect of Mannose-Binding Lectin (protein A) 1 (*MBL1*) Gene Polymorphisms on Milk Production Traits and Somatic Cell Count in Holstein Dairy Cattle

**J. Metin Kıyıcı[1,*], F. Daldaban[2], K. Arslan[2], B. Akyüz[2], M. Kaliber[1], M.U. Cinar[1,3]**

[1]*Department of Animal Science, Faculty of Agriculture, Erciyes University, Kayseri, Türkiye*

[2]*Department of Genetics, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Türkiye*

[3]*Department of Veterinary Microbiology & Pathology, Washington State University, Pullman, WA 99164, USA*

**ABSTRACT:** This study was conducted to determine polymorphisms (*rs479779108* G>A, *rs109492835* G>A, *rs453019355* T>C) in mannose-binding lectin-1 (*MBL1*) gene and to investigate their effects on milk yield (305 d, kg), milk composition (fat %, dry matter %, density g/cm$^3$, protein %, freezing °C, lactose %) and milk quality traits (somatic cell count, electrical conductivity and pH) in Holstein cows. For this purpose, 166 blood and 1660 milk samples of 166 Holstein cows in their 2$^{nd}$ lactation were used. All possible genotypes for the *MBL1* gene (*rs479779108* G>A - *Ava*II) (AA, AG, GG), *rs109492835* G>A *Mae*II (AA, AG) and *rs453019355* T>C *Hae*III (CC, TC, TT) were observed in the studied population. While the only *rs453019355* T>C polymorphisms was found to be associated with EC ($P<0.05$), all three SNPs (*rs479779108* G>A, *rs109492835* G>A, *rs453019355* T>C) were found to be associated with the SCC trait ($p<0.05$). In the examined population, *rs109492835* was found to be associated with density and protein, while *rs453019355* was found to be associated with fat, protein, freezing point, and milk yield characteristics.

***Keywords:*** *MBL1*; Polymorphism; Somatic cell count; Milk composition traits; Cattle

*Corresponding Author:*
Jale Metin Kıyıcı, Department of Animal Science, Faculty of Agriculture, Erciyes University, Kayseri, Türkiye
E-mail address: jalemetin@erciyes.edu.tr

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## INTRODUCTION

The mannose-binding lectin (*MBL*) which binds to various microorganisms and regulates the innate immune lectin-complement pathway, is a member of the collective protein family (Yang et al., 2018; Neth et al., 2000; Bouwman et al., 2006; Wang et al., 2012). Most mammals have two forms of *MBL*, *MBL*-A and *MBL*-C, which are encoded by two different genes, *MBL1* and *MBL2*, respectively. It has been reported that *MBL* deficiency is common in mammals (such as humans, mice, pigs, and cattle) and has a complex role in many diseases (Turner and Hamvas, 2000). The *MBL1* gene was mapped on cattle chromosome 28 (BTA28) and was located close to QTL related to immune related traits and production traits such as milk yield and milk fat content (Hu et al., 2022). These QTL could be caused by linkage disequilibrium with other adjacent genes, or they could be the consequence of pleiotropic effects of specific *MBL1* gene on physiological processes. *MBL1* gene possibly contributes to bacterial infection resistance and was proposed as a molecular marker for milk yield traits to control mastitis (Liu et al., 2011; Yuan et al., 2013).

Cattle with mastitis, an infectious condition affecting the mammary glands, experience an inflammatory response and consequently lower milk supply, which has a detrimental economic impact (Maity et al., 2020). Due to the disease's negative effects on milk supply and quality, rising health and control costs, waste milk, extra labor costs, and early culling, dairy farms suffer significant losses (Doehring and Sundrum, 2019). Mastitis shortens the productive lives of affected cows and lowers milk production and milk composition. Reductions in milk output, culling, and treatment costs make up 8%, 14%, and 78% of the total cost of mastitis losses, respectively. Since mastitis is impacted by a variety of local, regional, epidemiological, managerial, and economic factors, its economic impact should be estimated at the farm or herd level (Aqib et al., 2021; Kamaldeep et al., 2021). Worldwide, published estimates of the economic losses of clinical mastitis range from €61 to €97 per cow on a farm, with large differences between farms (Hogeveen et al., 2011). It has been reported (Ott, 1999) that the economic losses caused by subclinic mastitis in the United States dairy industry exceed $1 billion annually. In a study (Sarıözkan, 2019), it was stated that the loss per animal due to subclinical mastitis corresponds to 310 L ($100, 9.9% of lactation milk yield) and 710 L ($228, 22.6% of lactation milk yield) in clinical mastitis in Türkiye. The incidence of subclinical mastitis in Türkiye has been determined as 30%, and this rate varies according to regions (15-60%) (Akalın et al., 2016).

The most common indirect indicator for mastitis detection is the milk somatic cell count (SCC), which has moderate to significant relationships (r=0.30 to 0.80) with mastitis (Koivula et al., 2005; Sharma et al., 2011; Carlen et al., 2004; Chu et al., 2012). A SCC of more than 200,000 cells/mL is an indication of mammary gland infections, while less than 100,000 cells/mL indicates that the cow is uninfected (Pighetti and Elliott, 2011). Thus, increased levels of SCC are considered a sign of mastitis (Wang et al., 2019). SCC are accepted as features associated with mastitis resistance and their use against mastitis resistance continues all over the world. In addition, it has been stated that it is possible to use genomic selection as an effective and rapid method for screening genetically resistant dairy cattle susceptible to mastitis at a very early age (Khan et al., 2023). Wang et al (2011) stated in their study that a polymorphism in *MBL1* is also associated with SCC in dairy cows and that this gene may play a role in the host's response to mastitis.

Electrical conductivity (EC, mS/cm) of milk has been introduced as an indicator trait for mastitis. Mastitis vs EC correlation is varying between 0.65 and 0.8. EC is a cheap, simple, and instant method and thus, milk EC can be used as an alternative method for early diagnosis of mastitis (Norberg, 2005; Boas et al., 2017). In addition, positive correlations were reported in the literature between milk SCC and EC (Boas et al., 2017). One of the goals of genetic breeding in dairy cattle is to understand the genetic nature of the traits underlying milk yield and mastitis resistance (Magotra et al., 2019). The objective of the present study was to investigate SNPs in bovine *MBL1* gene and to evaluate the association of these polymorphisms with milk yield, milk composition and milk quality traits in Holstein dairy cattle.

## MATERIALS AND METHODS

The study has been approved by the local ethics committee at Erciyes University (dated 10.04.2013 and numbered 13/72).

### Animals and sample collection

The present study was conducted on 166 Holstein cattle, all of which were in the 2nd lactation, raised in a commercial dairy farm, Kayseri, Türkiye. In this study were used a total of 1660 milk samples, 10 samples from each cow. Cows were calved between November 2013 and January 2014 and milked routinely three times per day (7:00 am, 3:00 pm, 11:00 pm).

Milk composition traits (fat %, dry matter %, density (g/cm$^3$), protein %, freezing °C, lactose %), milk yield (305 d, kg) and milk quality (somatic cell count (SCC, (cells/mL)), electrical conductivity (EC, (mS/cm)) and pH data were recorded based on montly milking tests. A control day was specified in each month and 50 mL milk sample was taken from morning milking (07:00 am) of each animal in that control-test day. Samples were kept at +4 °C and used in milk composition analyses. Milk SCC measurements were made with DeLaval CC equipment (DeLaval, Stockholm, Sweden) somatic cell counter based on the fluorescent microscope cell counting technique. EC and pH measurements were made with Milkana Multi-Test milk analyzer (Mayasan, İstanbul, Türkiye).

**Collection of bovine blood samples and genetic analysis**

Blood samples were taken from the vena jugularis in cattle into 10 mL sterile vacuum EDTA tubes. DNAs were isolated from whole blood by phenol-chloroform-isoamyl alcohol (25:24:1) method (Sambrook et al., 1989). The primers for the PCR process applied to the isolated DNAs are given in Table 1 (Yuan et al., 2013). PCR reaction; 3 µL of DNA (50 ng/µL), a final volume of 20 µL was prepared by adding 2.0 mmol/L MgCl2, 0.25 mmol/L dNTP and 0.5 U Taq DNA polymerase containing 1X buffer [(NH4)2SO4 500 mmol/L]. PCR reaction; Initial denaturation at 95°C for 5 min, each cycle; binding for 30 seconds at 95 °C was completed by holding at 72°C for 7 min, followed by a total of 35 cycles of 30 seconds at 72°C. Genotyping of the obtained PCR products was done by Restriction Fragment Length Polymorphism (RFLP) process, and the cutting enzymes used in the process are given in Table 1.

Milk yield, milk composition and SCC, EC and pH values data were analyzed with a mixed model using the MIXED procedure of SAS v9.0 (SAS, Inc., Cary, NC, USA). The model included fixed effects of genotype, with random effects of control and sire. Genotype had 3 (*rs479779108*, *rs453019355*) or 2 (*rs109492835*) levels according to genotype of the investigated genes. Control represented the test day in which test data for milk traits were recorded once a month in a lactation period (305 days) and it has 10 levels and sire had 19 levels. SCC data were subjected to log10 transformation to make the data normally distributed.

## RESULTS

In the study, three genotypes (AA, AG, and GG) in *rs479779108* G>A, two genotypes (AA and AG) in *rs109492835* G>A and three genotypes (TT, TC and CC) in *rs453019355* T>C were determined in Holstein cows. The electrophoresis patterns of *MBL1* and DNA ladder (100 bp) are presented in Figure 1.

Through PCR-RFLP three allelic variants corresponding to the G~A mutation at *rs479779108* in intron 1, G~A mutation at *rs109492835* and T~C mutation at *rs453019355* in exon 2 of bovine *MBL1* gene could be detected, respectively.

The Chi-square test result (at one degree of freedom at 1% level) are presented in Table 2 revealing Hardy-Weinberg equilibrium for *MBL1* gene *rs479779108* G>A, *rs109492835* G>A, *rs453019355* T>C polymorphisms in Holstein cows. The most common genotype was determined as AG in SNPs *rs479779108* G>A, AA in SNPs *rs109492835* G>A and TC in SNPs *rs453019355* T>C. Gene and genotypic frequencies of *MBL1* (*rs479779108* G>A, *rs109492835* G>A, *rs453019355* T>C) gene determined by PCR-RFLP in Holstein breed cows are given in Table 2.

The least squares mean and standard errors of three SNPs (*rs4797791082* G>A, *rs109492835* G>A, *rs453019355* T>C) belonging to the *MBL1* gene and milk yield (305 d, kg) and milk composition traits are given in Table 3.

**Table 1.** SNPs, regions, primers, annealing, PCR base lengths, genotypes and restriction enzymes.

| SNP/ location | Primer Sequence | AT (°C) | SAF (bp) | Possible genotypes | RE |
|---|---|---|---|---|---|
| c.1252 Int-1 G>A<br>*rs479779108* | F: ACCTTGGGTCACCTGCAACAG<br>R: GGTAGTTTAGGCAGCCCTAAAGC | 62.5 | 226 | GG: 193,33<br>GA: 226, 193, 33<br>AA: 226 | *Ava*II |
| c.2534 Ex-2 G>A<br>*rs109492835* | F: GTATCCTTCTCAAATACAAAAGAC<br>R: CCCCTGTCTCTATGCTAGAC | 52.5 | 217 | GG: 194, 23<br>GA: 217, 194, 23<br>AA: 217 | *Mae*II |
| c.2569 Ex-2 T>C<br>*rs453019355* | F: GTGGTGGCAAATGTTGGCTAAAC<br>R: TGGCTCTCCCTTTTCTCCCTT | 63.5 | 255 | TT: 255<br>TC: 255, 178, 77<br>CC: 178, 77 | *Hae*III |

SAF: Size of amplification fragment; AT: Annealing temperature; RE: Restriction enzyme.

The least squares mean and standard errors for SCC, EC and pH values of three SNPs (*rs479779108* G>A, *rs109492835* G>A, *rs453019355* T>C) belonging to the *MBL1* gene are given in Table 4.

The value of milk SCC for individuals with *MBL1 rs479779108*-AA, *rs109492835*-AA and *rs453019355*-CC genotypes had lower SCC values than individuals with other genotypes.

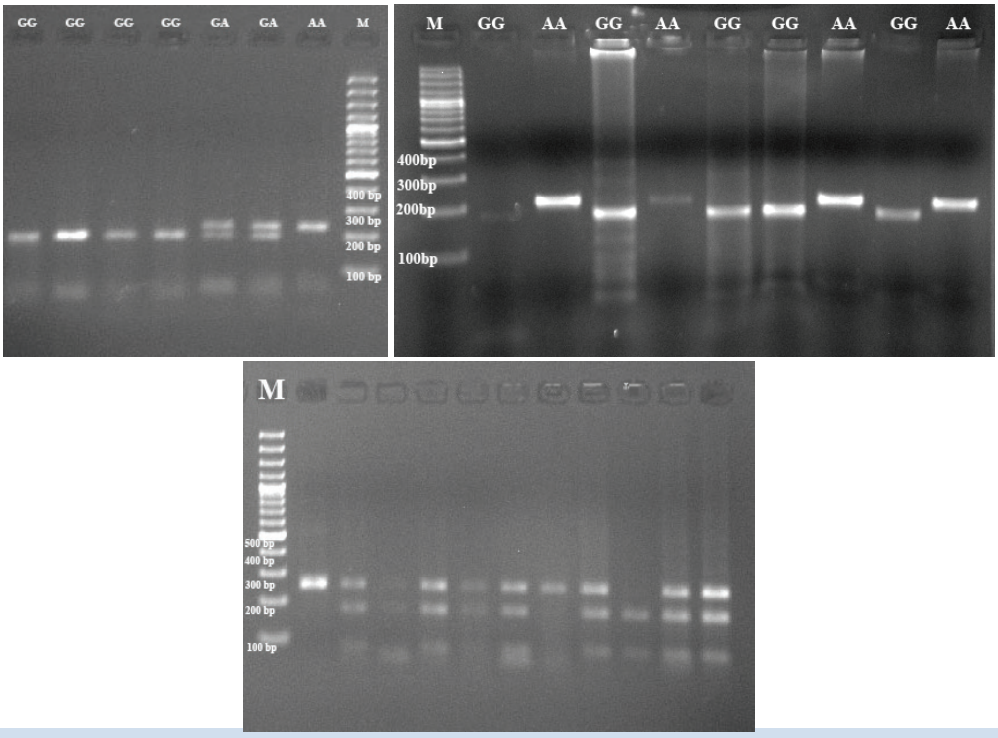


**Figure 1.** Agarose electrophorese pattern of *MBL1* gene SNPs [*rs479779108* G>A (a), *rs109492835* G>A (b) and *rs453019355* T>C (c)] M: DNA ladder (100 bp).

**Table 2.** Genotype and allel frequencies of *MBL1* (*rs479779108* G>A, *rs109492835* G>A, *rs453019355* T>C) gene determined by PCR-RFLP in Holstein breed cows.

| Gene | n | Genotype Frequency | | | Allel Frequency | | $\chi^2$ | *p* |
|---|---|---|---|---|---|---|---|---|
| rs479779108 | 166 | **AA (Obs)** | **AG (Obs)** | **GG (Obs)** | **A** | **G** | 13.918 | 0.000 |
| | | 0.08 (14) | 0.61 (102) | 0.30 (50) | 0.39 | 0.61 | | |
| rs109492835 | 166 | **AA (Obs)** | **AG (Obs)** | **GG (Obs)** | **A** | **G** | 166 | 0.000 |
| | | 0.77 (128) | 0 (0) | 0.23 (38) | 0.77 | 0.23 | | |
| rs453019355 | 166 | **TT (Obs)** | **TC (Obs)** | **CC (Obs)** | **T** | **C** | 51.499 | 0.000 |
| | | 0.10 (15) | 0.78 (129) | 0.13 (22) | 0.53 | 0.58 | | |

n: Number of animals; Obs: Observed genotype; Exp: Expected genotype; $X^2$: Chi-square

**Table 3.** The least-square means and standard errors for milk yield (305 d, kg) and milk composition traits values of three SNPs (*rs479779108* G>A, *rs109492835* G>A, *rs453019355* T>C) of the MBL-1 gene.

| SNPs | Genotypes | Traits | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **Fat (%)** | **Dry Matter (%)** | **Density (g/cm$^3$)** | **Protein (%)** | **Freezing (°C)** | **Lactose (%)** | **Milk Yield (305 d, kg)** |
| *rs479779108* | AA | 3.23±0.13 | 9.02±0.05 | 30.82±0.30 | 3.42±0.07 | -0.59±0.03 | 4.97±0.03 | 8120.0±205.4 |
| | AG | 3.16±0.08 | 9.10±0.03 | 31.12±0.16 | 3.42±0.03 | -0.59±0.02 | 4.99±0.01 | 7774.0±87.6 |
| | GG | 3.22±0.09 | 9.07±0.03 | 30.99±0.19 | 3.47±0.04 | -0.59±0.02 | 4.99±0.02 | 7825.8±112.4 |
| *rs109492835* | AA | 3.20±0.08 | 9.08±0.02 | 30.97±0.15$^b$ | 3.42±0.03$^b$ | -0.59±0.02 | 4.99±0.01 | 7820.5±80.1 |
| | AG | 3.08±0.10 | 9.12±0.03 | 31.44±0.21$^a$ | 3.52±0.05$^a$ | -0.60±0.02 | 5.01±0.02 | 7783.4±131.6 |
| *rs453019355* | CC | 3.32±0.11$^a$ | 9.03±0.04 | 30.70±0.25 | 3.40±0.06$^b$ | -0.59±0.03$^a$ | 4.96±0.01 | 8897.4±160.5$^a$ |
| | TC | 3.13±0.08$^b$ | 9.10±0.03 | 31.11±0.16 | 3.41±0.03$^b$ | -0.59.±0.02$^b$ | 5.00±0.01 | 7650.2±80.5$^b$ |
| | TT | 3.47±0.13$^a$ | 9.08±0.05 | 31.19±0.30 | 3.66±0.07$^a$ | -0.59±0.03$^{ab}$ | 4.98±0.03 | 7468.5±197.8$^b$ |

$^{a,b}$The means indicated with different letters in the same column are significantly different ($p<0.05$)

**Table 4.** Least squares mean and standard errors for SCC, EC and pH values of three SNPs (*rs479779108* G>A, *rs109492835* G>A, *rs453019355* T>C) of the *MBL1* gene.

| Gene | Genotypes | SCC (Log10SCC) | EC (mS/cm) | pH |
|---|---|---|---|---|
| rs479779108 | AA | 4.84 ± 0.07[a] | 4.96 ± 0.05 | 6.88 ± 0.05 |
| | AG | 5.18 ± 0.04[b] | 5.02 ± 0.03 | 6.89 ± 0.01 |
| | GG | 5.09 ± 0.05[c] | 5.04 ± 0.04 | 6.89 ± 0.01 |
| *rs109492835* | AA | 5.10 ± 0.04[a] | 5.03 ± 0.03 | 6.89 ± 0.01 |
| | AG | 5.24 ± 0.05[b] | 5.02 ± 0.04 | 6.89 ± 0.01 |
| rs453019355 | CC | 4.94 ± 0.06[a] | 4.95 ± 0.04[a] | 6.87 ± 0.01 |
| | TC | 5.15 ± 0.04[b] | 5.04 ± 0.03[b] | 6.89 ± 0.01 |
| | TT | 5.24 ± 0.07[b] | 5.01 ± 0.05[b] | 6.89 ± 0.02 |

[a,b,c]The means indicated with different letters in the same column are significantly different (p<0.05)

## DISCUSSION

In the current study, the determination of polymorphisms (*rs479779108* G>A, *rs109492835* G>A, *rs453019355* T>C) in *MBL1* genes in Holstein dairy cattle and its effect on milk production, milk composition and milk quality were investigated. In the study significant relationship was observed between *MBL1* gene polymorphisms (*rs479779108* G>A, *rs109492835* G>A, *rs453019355* T>C) and milk SCC. Similar studies, also, reported significant association of SNPs in *MBL1* with milk SCC, an important phenotypic indicator of bovine mastitis (Liu et al., 2011; Koivula et al., 2005; Khan et al., 2023; Wang et al., 2011; Moretti et al., 2021; Zhao et al., 2012). In the study, milk SCC values were significantly lower in individuals with AA genotype in *rs479779108* SNPs, AA genotype in *rs109492835* SNPs, and genotype CC in *rs453019355* SNPs. In a similar study by Magotra et al (2019), it was reported that animals with GG genotype for 2534 G>A SNP had dear SCC (Hardhenu cattle 4.50±0.36, Murrah buffalo 3.34±0.19). In a similar study, Yuan et al. (2013) a significant correlation milk SCC was detected in c.2534 G>A, and they stated that the value of milk SCC for individuals with genotype GG was significantly lower than those of genotype GA and AA. Baghel et al. (2022) was stated with milk yield traits and SCS a significant difference among g.2686T>C genotypes with SCS in Hariana cattle only. On the other hand, Baghel et al. (2023) found no significant difference for any of the traits between the SNPs and SCS they examined in their study on the molecular characterization of the *MBL1* gene in Indian buffaloes.

Among the SNPs examined in the study, only the relationship between *MBL1 rs453019355* T>C and electrical conductivity (EC) was found to be significant, but this relationship was not found to be significant for the other SNPs (*rs479779108* G>A and *rs109492835* G>A). Theoretically, due to the positive correlation between SCC and EC, genotypes with low SCC are expected to also have low EC. In the study, the lowest EC value in *MBL1 rs453019355* T>C polymorphism was determined in the CC genotype, as in SCC.

Among the SNPs examined in the study, the relationship between *MBL1 rs453019355* T>C and milk fat, milk protein, freezing point and *MBL1 rs109492835* G>A and density and milk protein was significant (P<0.05). Among the SNPs examined in the study, only the relationship between *MBL1 rs453019355* T>C and milk yield was found to be significant (P<0.05), and individuals with the CC genotype had higher milk yield than animals with the TC and TT genotypes. Wang et al (2011) stated in their study in Chinese Holstein populations that no significant correlation was observed between each of the three SNPs of the *MBL1* gene and milk production characteristics (fat content, protein content and 305 d milk yield). Similarly, Baghel et al (2023) stated that there was no significant difference between the *MBL1* gene and milk yield traits for any trait.

Incidence of mastitis is targeted to be reduced mastitis prevention programs of dairy cattle breeding. Genetic selection is widely used for this purpose (Kiyici et al., 2022). Studies of the inheritance of mastitis resistance have revealed that this heritability was quite low (0.10-0.16) (Khan et al., 2023). Therefore, studies in the creation of mastitis-resistant genetic material have focused on the use of SCC related genes (Kiyici et al., 2022; Metin Kiyici et al., 2020). The use of somatic cell count (SCC) and somatic cell score (SCS) as correlated traits in the indirect selection of animals against mastitis resistance is in progress globally (Wang et al., 2011).

## CONCLUSION

The results of this study added new evidence that *MBL1* is an important candidate gene for the selection of dairy SCC for dairy mastitis resistance traits and that these SNPs can be used as markers for improving mastitis resistance in cattle. Further studies are expected to support the hypothesis that *MBL1* may have

a functional role influencing dairy mastitis.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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