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The variation in the beta-casein genotypes and its effect on milk yield and genomic values in Holstein-Friesian cows

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ABSTRACT

The A2 milk marker is gaining popularity worldwide; thus, many farms plan to convert their dairy cattle herds to the A2A2 genotype. Variation in beta-casein genotypes needs to be monitored in large dairy cattle populations. Therefore we aimed to evaluate the genotypic distributions, population genetics, and diversity parameters in Holstein-Friesian cows. A total of 1200 cattle were genotyped using the Affymetrix® Axiom® array system. We performed an association analysis regarding the *CSN2* genotypes and phenotypic traits, including lactation and test-day milk yield. We next evaluated the effects of the genotypes considering the genetic merit of the animals. Animals were grouped based on their PTAs for milk production, fat, protein, and daughter pregnancy rate. Thus, we tested the genotype × genetic merit interaction for significance. The A2 allele frequency is remarkably high (0.68), and the heterozygous genotype is predominant (46.25%). The marker showed intermediate variability and diversity levels, indicating a considerable frequency of the A1A1 genotype (9.33%) remains in the population. ANOVA results showed no significant association between the *CSN2* genotypes and milk yield traits. A similar finding is valid for the genotype × genetic merit regarding the genomic test results. The data presented here may be helpful for further investigations and applications on A2 milk production.

KEYWORDS

A2 milk; *CSN2* gene; genomic test; Holstein cattle; milk production

Introduction

Cow's milk is vital in human nutrition because it is an excellent source of protein, fat, carbohydrates (mainly lactose), and micronutrients. Bovine milk contains 3.5–4% protein.[1] Milk protein can be principally divided into two categories based on its solubility: soluble proteins (whey proteins) and insoluble proteins (caseins), which constitute ∼80% of the total bovine milk proteins.[2] Casein is a family of phosphoproteins that are the major proteins in milk, specifically from mammals, including bovines. These proteins are known to be precursors of several different bioactive peptides and play a crucial role in the nutritional value of milk and the production of dairy products, such as cheese and yogurt.[3]

The variation of casein has been widely studied, as it can significantly impact the functionality and quality of milk and dairy products. The bovine casein genes have been the subject of many studies aimed at understanding genetic variation and its effects on milk production traits, milk quality/technological properties, and human health.[1,4–6] Bovine casein's best-known genetic variations differ by the A1 and A2 variants, which differed by a single-nucleotide polymorphism (SNP) at the 67th amino acid position. The mutation in the gene coding for β-casein [g.8101C > A (p.His67Pro)] has been designated as the alteration from CCT on the A1 allele to CAT on the A2 allele.[7] This is a missense mutation that leads to the production of mutant β-casein by changing its proline amino acid to histidine.[7,8] This amino acid alteration causes the *in vitro* and *in vivo* digestion patterns of the resulting peptide chain to change and the release of beta casomorphin 7 (BCM7), which has opioid properties.[1,9] BCM7 is enzymatically released from β-casein A1 upon the action of an alternative proteolytic pathway through gastrointestinal enzymes in humans.[9] Concerning this genetic difference, bovine milk can be categorized into

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two subgroups, including 'A1 milk' containing genetic variants of β-casein with histidine, and 'A2 milk' containing variants with proline in position 67.[1,6,9,10] The effect of consuming raw, processed cow's milk or dairy products, such as cheese containing these different beta-casein variants on human health is highly controversial. In 2009, the European Food Safety Authority (EFSA) presented a scientific report about the potential impact of BCMs and related peptides on human health. In this context, EFSA announced that there is no clear evidence of a direct relationship between BCM7 and the development of certain non-transmissible diseases.[11] Here, it should be noted that this comprehensive report drew attention to the fact that casomorphins can cause gastrointestinal disorders and intolerance-like symptoms and the need to deepen scientific studies examining the effects of the A1 variant on disease development or worsening of symptoms.[9] However, in the decade after this report, the interest in A2 milk has increased, and many *in vitro* and *in vivo* studies have been published on this subject. Many studies pointed out an association between the presence of milk-derived BCM7 and a higher incidence of many human diseases, including diabetes mellitus,[12] autism,[13] sudden infant death syndrome,[14] gastrointestinal problems,[15] some neurological, neurodevelopmental, and mental disorders.[16] It is worth noting that there are also studies reporting that there is not enough cumulative scientific evidence to suggest that the A1/A2 genotype difference is directly related to human health.[6,9,17]

Conventionally marketed milk contains both β-casein A1 and A2 variants and it is obtained from A1A1 and A2A2 homozygotes and A1A2 heterozygous cows. On the other hand, A2 milk (A1-free) and its products are commercially available in several countries/regions, including New Zealand, North America, Australia, China, the United Kingdom, the Netherlands, and Italy.[1,18] Although studies related to human health are quite intriguing, one of the crucial points of A2 milk production that cannot be ignored is the level of dairy cow farms. Recently, breeder associations are promoting interest in A2 milk production,[2] but there are clues indicating that cows carrying the A1 variant exhibit higher reproductive performance than those carrying the A2 variant.[18–20] Furthermore, some studies have reported that the A2A2 genotype provides higher milk yield.[18,21,22] A recent study indicated that the highest fat percentage was found for the genotype A1A1 compared to the genotype A2A2 which demonstrates a negative impact on milk composition.[6] However, many studies point out that there is no significant relationship between *CSN2* gene variants and milk yield and content and that analyses in larger populations are necessary. Although some comprehensive analyses exist, there are limitations in the studies conducted on genetic variation in the bovine *CSN2* and A2, namely: (1) Genetic variation has been evaluated in a limited number of animals. (2) Phenotypic and genotypic data from several farms with different environmental conditions have been considered (although the farm effect is added to the statistical models). (3) In some cases, genotyping has been performed in populations with insufficient number of sires, resulting in low genetic variation. Therefore, the present study was performed to evaluate the genetic variation in the bovine *CSN2* gene in a relatively large Holstein-Friesian cow population concerning A1/A2 allele discrimination to analyze the association between the corresponding genotypes and milk yield.

Materials and methods

Animals and sampling

A total of 1200 purebred Holstein-Friesians raised in a commercial herd located in the Marmara region of Turkey were used in this study. Daily milk yield was recorded individually in milking parlors equipped with electronic devices that automatically record the quantity of milk. All cows were housed in free-stall barns with the same management procedures. They were milked three times a day and fed ad libitum with corn silage, alfalfa hay, oat hay, and pellet-based dairy total mixed ration that was formulated to meet NRC 2001[23] recommendations. The entire population included 1200 cows but animals with missing sire identification, incomplete milk yield records, incorrect calving dates, and age at calving were excluded from the analysis. We next evaluated the pedigree data to select unrelated individuals. Taken together, from the total population, we analyzed milk yield records of 252 unrelated cows from the first and second lactation. Bursa Uludag University Local Ethics Committee for Animal Research approved the study (approval number: 2022–15/02).

Genotyping

Genomic DNA was extracted from ear tissue samples by Genetic Visions Rapid DNA preps with K buffer (Genetic Visions, Middleton, USA). DNA quantity (ng/μL) and purity (260/280 adsorbance value) were assessed by the NanoDrop 2000c Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Samples

were genotyped using the Affymetrix® Axiom® array (Affymetrix, Santa Clara, CA, USA) incorporation into a custom 70 K high-density SNP Array in a GeneTitan® Multi-Channel platform (Affymetrix). Data analysis was performed following the Axiom® Genotyping Solution Data Analysis Guide (Rev4).

Evaluation of genetic merit

We evaluated the genetic merit of the 1200 cattle by computing direct genomic values (DGV) and genomic predicted transmitting ability (GPTA) values for economically important dairy traits. Vision20® test (Genetic Visions-ST, Middleton, WI, USA) results were generated based on the Quick Turnaround Genomic Predictions (QTGPs). Furthermore, we evaluated EcoFeed® and Type production index (TPI®) values. EcoFeed® is calculated by ST Genetics and is a registered trademark. TPI® is a comprehensive breeding index and a registered service mark developed by the USA Holstein Association. Predicted transmitting ability (PTA) values of milk (PTA milk), fat (PTA fat), and protein (PTA Pro) were computed for each cow. Variations in genomic predictions for PTA milk, PTA fat, PTA Pro, and PTA for daughter pregnancy rate (DPR) are presented in Fig. S1. We also evaluated PTA levels in cows with milk yield records selected from the total population of genomically tested cows based on the *CSN2* genotypes. The distributions according to genotypes for PTA milk, fat, protein, and DPR in cows where the effect of *CSN2* on milk yield was examined are presented in Fig. 1. Data of the TPI and Net merit (NM$) of the studied population are provided in Table S1.

Population genetics analysis

The allelic and genotypic distribution was calculated by the standard procedure.[24] Hardy-Weinberg equilibrium (HWE) was tested by evaluating the significance of the difference between the theoretical and experimental frequencies of the genotypes using the χ^2 test. The genotype structure of the cattle population and the strength of the genetic marker were assessed by estimating the population genetics parameters and diversity indices. In this context, the theoretical heterozygosity (He_{the}), expected homozygosity (Ho), the fixation index (F_{IS}), level of possible variability realization (*V*%), the number of effective alleles (Ne), and the polymorphism information content (PIC) were calculated.

We also evaluated the diversity indices, including the Shannon-Weaver diversity index (*H'*), Simpson dominance index (D_2), and the Gini coefficient (*GC*), by using relevant equations. All details of the calculations and equations for the population genetics and diversity parameters are presented in the Supplementary Materials and Methods.

Association analysis

The Minitab software (Minitab, Pennsylvania, USA, v17.1.0) was used to analyze the relationship between the *CSN2* genotypes and milk production. The data were tested by Anderson-Darling for normality. The general linear model (GLM) procedures and the least squares mean (LSM) adjustment were used. The statistical model included the effects of lactation number, calving season, and days in lactation. A probability value of <0.05 was considered significant. As shown in the following statistical model, lactation number (first or second lactations) and calving season (autumn, winter, spring, and summer) were added to the model as fixed effects, while days in lactation were added as a covariate.

$$Y_{ijklm} = \mu + A_i + B_j + C_k + \beta D_l + e_{ijklm}$$

where: Y_{ijklm} = the studied traits; μ = the overall mean; A_i = the fixed effect of calving season (i = autumn, winter, spring, and summer); B_j = the fixed effect of lactation number ($j = 1^{st}$ and 2^{nd}); C_k = the fixed effect of *CSN2* genotypes (k = A1A1, A1A2, and A2A2); βD_l = the regression effect of days in lactation; and e_{ijklm} = the random residual effect.

We next evaluated the effects of interaction through the *CSN2* genotypes and genomic predictions provided by GPTA values for milk production, fat, protein, and DPR. Based on the median PTA milk values of the 252 cows, phenotyped cows were grouped as having above or equal *vs.* below-median milk PTA. The interaction was then added to the statistical model (presented above) as a fixed effect and tested for significance. The same analysis was performed for GPTA values of milk fat, protein, and DPR. Descriptive statistics of the PTA values genomically tested and phenotyped cows are presented in Table S2. The comparison of the *CSN2* genotypes based on the TPI and NM$ values was performed using the one-way analysis of variance (ANOVA) and Tukey's multiple-comparison test.

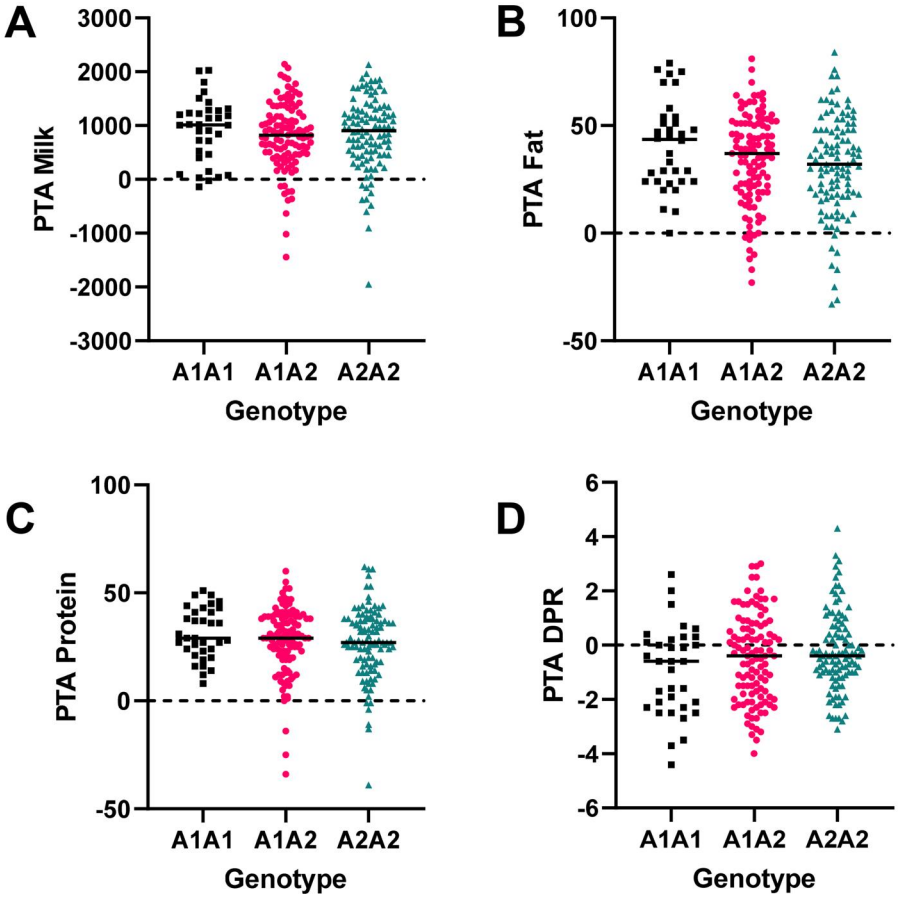


Figure 1. Distribution of PTA values according to the *CSN2* genotypes including A1A1, A1A2, and A2A2 in the phenotyped cows ($n = 252$). (A) PTA milk, (B) PTA fat, (C) PTA protein, and (D) PTA DPR (daughter pregnancy rate).

Results

Genetic variation in the bovine *CSN2* gene

Table 1 shows the genotypic and allelic frequencies for the bovine beta-casein gene in Holstein-Friesian cattle. Among the 1200 Holsteins, the frequency of the heterozygous genotype was high (46.25%). A considerable A2A2 genotype frequency was observed in the population. Relatively high frequencies of the A2A2 and heterozygous genotypes resulted in a remarkably high A2 allele frequency (0.68). The χ^2 test indicated that the genotypic distribution of *CSN2* was in agreement with HWE expectations ($p > 0.05$). The population genetic indices for the *CSN2* gene of Holstein cows are also presented in Table 1. The fact that heterozygotes constitute the majority of the population caused the F_{IS} value to be low (−11.75) and the *V*% value to remain at an acceptable level (~43%). Population genetics and diversity parameters indicated an intermediate level of genotypic variation for the bovine *CSN2* gene in the studied population (Table 1).

Table 1. Genotype and allele frequencies, population genetics parameters, and genetic diversity indices in bovine beta-casein gene concerning A1/A2 alleles ($n = 1200$).

Locus	CSN2		
Genotypes	A1A1	A1A2	A2A2
n	112	555	533
Genotype frequency (%)	9.33	46.25	44.42
Expected genotype frequency[a]	10.53	43.85	45.62
Alleles	A1		A2
Alele frequency	0.32		0.68
Theoretical heterozygosity (H_{the})	0.4352		
Experimental heterozygosity (H_{exp})	0.4625		
Homozygosity (Ho)[b]	0.5648		
Fixation index (F_{IS})	−11.7527		
Level of possible variability realization (PVR)	0.4344		
Number of effective alleles (Ne)	1.7705		
Polymorphism information content (PIC)	0.3405		
χ^2 (HWE)	3.6080		
P^c	0.0575		
Shannon-Weaver diversity index (*H′*)	0.9827		
Simpson dominance index (*D*)[d]	2.5110		
Gini coefficient (*GC*)	0.2145		

CSN2: beta casein; *n*: number of experimental cows; χ^2 (HWE): Hardy–Weinberg equilibrium χ^2 value.
[a]The expected genotype distribution according to HWE.
[b]In diallelic loci, 1 − theoretical heterozygosity (H_{the}) = locus homozygosity (Ho).
[c]$p > 0.05$—consistent with HWE.
[d]Reciprocal Simpson index values were calculated.

Table 2. Least squares means ± standard errors and levels of significance for the effect of bovine beta-casein genotypes on milk yield in Holstein cows ($n = 252$).

	Genotypes			
Trait	A1A1	A1A2	A2A2	*p*-Value
Lactation milk yield	8283 ± 347	8611 ± 228	8323 ± 256	0.411
Test day milk yield	36.37 ± 1.18	36.47 ± 0.79	35.89 ± 0.88	0.788

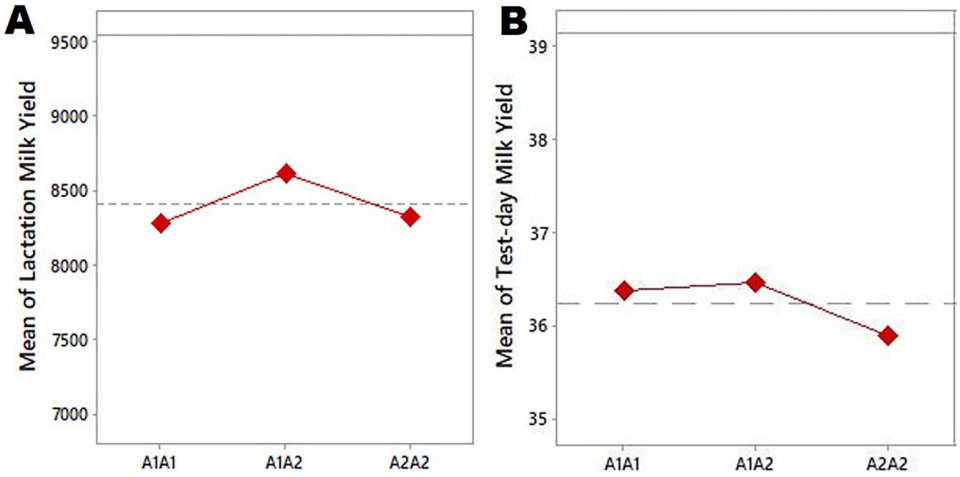


Figure 2. Factorial plots of the associations observed in the general linear model (GLM) regarding the bovine *CSN2* genotypes (A1A1, A1A2, and A2A2). (A) The main effects plot for the effects of *CSN2* genotypes on lactation milk yield. (B) The main effects plot for the effects of *CSN2* genotypes on test-day milk yield.

Marker effects on phenotypic trait means

The results of the association analysis between *CSN2* genotypes and milk yield are presented in Table 2. Although the heterozygous animals were characterized by higher lactation milk yield compared to A1A1 and A2A2 homozygotes, this relationship was not substantiated in the multiple comparisons ($p > 0.05$). Similarly, we observed that the test day milk yield means of different genotypes were quite close to each other (Fig. 2). Hence, it can be interpreted that there was no statistically significant difference between the genotypes regarding milk yield in the studied Holstein-Friesians. Concerning interaction through the *CSN2* genotypes and PTA values for milk, fat, protein, and DPR, no statistical significance was observed in the ANOVA for milk production traits. The least squares means for the *CSN2* × genetic merit interaction are presented in Table 3.

Discussion

In dairy herds aiming to produce A2 milk, a necessary culling process is required for effective implementation, while using A2 genotyped semen can help reclaim heterozygotes to obtain A2 calves. On the other hand, the A2 milk production must be separated from the milk of A1 cows. To prevent A1 contamination, the cows must be milked separately in separate tanks. Milk should be transported independently and processed individually in the factory.[18] Ultimately, providing adequate selection and management in the farm and finding a large potential market with a fair milk price is indispensable to make the whole process profitable at both farm and cow production levels. It is important to note that in countries where exchange rates are high and national biotech firms are limited (such as Turkey), genetic analysis still burdens farms considerably. In Turkey, no national program for breeding Holstein-Friesian cattle is implemented regarding *CSN2* genotyping. However, the worldwide popularity of A2 milk encourages breeders to try to convert their herds to A2, thus applying A2 genotyped semen and A1A1-culling practices.

We screened for *CSN2* genotypes (A1A1, A1A2, and A2A2) in a considerably large Holstein population ($n = 1200$), and we next evaluated the relationship of these *CSN2* genotypes to milk production. Our findings suggest a frequency of the A1 allele (0.32) in the population. Recent studies have shown that the average prevalence of the A1 allele in European, American, and Australian dairy cattle populations is 0.35, while the A2 allele is 0.61.[16] It is known that

Table 3. Least-squares means, and standard errors for the effects of genotype × genetic merit interaction on lactation milk yield and test-day milk yield in Holstein-Friesian cows.

	Lactation milk yield (kg)			
Group[a]	*CSN2* genotype × PTA milk	*CSN2* genotype × PTA fat	*CSN2* genotype × PTA protein	*CSN2* genotype × PTA DPR
A1A1-G1	8274 ± 451	8277 ± 463	8367 ± 452	8308 ± 433
A1A1-G2	8230 ± 464	8280 ± 457	8174 ± 462	8261 ± 486
A1A2-G1	8737 ± 261	8729 ± 295	8643 ± 284	8587 ± 299
A1A2-G2	8389 ± 318	8506 ± 273	8567 ± 284	8648 ± 269
A2A2-G1	8279 ± 291	8459 ± 295	8439 ± 285	8637 ± 340
A2A2-G2	8337 ± 351	8130 ± 337	8081 ± 362	8111 ± 295
	$p = 0.708$	$p = 0.892$	$p = 0.854$	$p = 0.479$
	Test-day milk yield (kg)			
A1A1-G1	36.92 ± 1.63	37.88 ± 1.68	36.76 ± 1.64	37.34 ± 1.45
A1A1-G2	35.74 ± 1.56	35.15 ± 1.53	36.03 ± 1.56	35.02 ± 1.76
A1A2-G1	36.89 ± 0.92	36.75 ± 1.03	36.75 ± 1.01	37.06 ± 1.03
A1A2-G2	35.80 ± 1.09	36.36 ± 0.95	36.28 ± 0.98	35.87 ± 0.95
A2A2-G1	36.28 ± 1.01	35.94 ± 1.02	35.55 ± 1.01	35.07 ± 1.15
A2A2-G2	35.20 ± 1.20	35.97 ± 1.19	36.53 ± 1.22	36.49 ± 1.04
	$p = 0.768$	$p = 0.539$	$p = 0.660$	$p = 0.194$

PTA: predicted transmitting ability; DPR: daughter pregnancy rate.
[a]Assessment of the effects of *CSN2* × genomic value interaction on milk production. Based on the median PTA values, phenotyped cows were grouped as having above or equal median (G1) *vs.* below-median (G2) to evaluate the effects of the *CSN2* genotypes (A1A1, A1A2, and A2A2) × genetic merit of cows on the phenotypic traits.

breed is a crucial factor in the evaluation of the A1/A2 allele distribution in dairy cattle. The A1 allele frequency is relatively high in Ayrshire and Red Denmark breeds (0.51–0.72), while Guernsey, Jersey, Brown Swiss, and Brown Italian cattle are characterized by low A1 frequency (0.04–0.14).[16] Considering the most famous dairy cattle breed, Holstein-Friesian, results can vary considerably. A recent study by Miluchova et al.[6] indicated that breeding in high-producing herds alters the population's genetic structure. These authors reported that an increase in the frequency of the A2A2 genotype (57.64%) was observed in the analyzed Slovak Holstein cow population. They also indicated that the presence of a selection program based on the A2A2 genotype directly affects the A2 allele frequency as expected. Therefore the A2 allele frequency was seen to increase up to 80.30% in one of the studied herds that implemented a selection to increase the proportion of A2A2 cows.[6] In general, the frequency of the A1 variant ranges from 0.25 to 0.51 in Holstein-Friesian cattle.[2,16,22,25] It is essential to note that there are also results in which the A1 frequency is higher than this range.[20] In fact, the A2 allele is known to be the oldest variant compared to the others that originated via mutation.[7,16] Although more supporting scientific evidence is still needed to launch A2 milk as healthy milk, the demand for A2 milk, based on its popularity worldwide, has encouraged many breeders to select A2A2 cows in dairy cattle herds. In some cases, increased frequency of the A2 allele at the herd level in many farms where A2 milk production is not an aim unintentionally due to the use of bulls with the A2A2 genotype. Needless to say, if the adverse effects of A1 beta-casein on human health are confirmed by detailed and controlled *in vitro* and *in vivo* experiments, consumer demand for A2 milk will increase considerably. Thus, selection based on the bovine *CSN2* gene will be more stringent.

Population genetic parameters provide precious information in genetic studies, especially in analyses where the variation of a specific genetic marker is examined. It is also essential to investigate the genotypic distribution of the considered marker in dairy cattle exposed to intense selection pressure, such as Holstein-Friesians.[26] One of the most decisive indices to assess the population's genetic structure is heterozygosity which is proportional to the genetic variability at a locus. It is suitable for theoretically considering the effect of limited population size on genetic variation.[6] A remarkable number of heterozygotes ($n = 555$) remains in our study population. Expected and observed genotype frequencies indicated that the genotype distribution agrees with HWE expectations ($p > 0.05$). Furthermore, F_{IS} and V% values showed that genetic variability was maintained despite the selection in the experimental farm (Table 1). The PIC is a robust parameter that enables the evaluation of the variation and understanding of the informativeness of the selected markers in a particular population.[26,27] We calculated the PIC for the *CSN2* marker as 0.3405, which indicates a moderately informative polymorphism based on the suggestions by Botstein et al.[28]. The marker also exhibited an acceptable level of Ne (~1.77). These results confirmed that admissible genetic variability and fairly

balanced genotypic distribution in the *CSN2* resulted in adequate population genetic indices values in the tested Holstein herd. This interpretation was substantiated by evaluating the diversity parameters (H', D, and GC), as shown in Table 1. Diversity parameters can describe variation at multiple levels of genetic organization, from SNPs through whole species or larger taxonomic units to ecosystems.[29] Concerning the *CSN2* A1/A2 variant discrimination, we estimated acceptable levels of diversity. It can be asserted that a high proportion of heterozygotes (46.25%) led to the calculation of desired values for the population genetics and diversity indices. Here it should be noted that the observed genetic variation in this study is no longer preferred if a distinctive A2 selection will be chosen in this flock. In this context, procedures for converting heterozygotes to A2A2 should be considered. The solution is either culling heterozygotes from the herd or using A2-genotyped semen. In fact, bovine *CSN2* A1/A2 allele separation applications clearly demonstrate how valuable and effective genetic selection procedures can be and how flexible they can be according to consumer demand, economic dynamics, and current requirements.

Another critical issue to be monitored in dairy cattle selection programs to be made based on the A2A2 genotype is milk production. It is well known that genes that determine quantitative phenotypic traits have pleiotropic characteristics. The farmers should take appropriate steps to allow for a systematic reduction in the number of cows and bulls with the A1 allele and consequently reduce the spread of this undesirable allele in a dairy cattle population. At this point, the question of how milk yield, content, and reproductive characteristics will be affected is also of great value. Hence, we investigated the effects of g.8101C > A (p.His67Pro) alteration on lactation milk yield and test-day milk yield in high-yielding Holstein-Friesian cows. Although the mean lactation milk yield of A1A1 cows seemed to be lower, ANOVA results revealed no statistically significant difference among *CSN2* genotypes (Table 2). The test-day records are considered a more accurate expression of the cattle milk production as a full lactation data expression.[6] Concerning test-day milk yield, the means of each genotype were very close to each other, and no statistical difference was observed in this study. Previous studies revealed different results on the effects of *CSN2* genotypes on milk yield and content. Many researchers reported that the A2 genotype is associated with desired milk production traits. Soyudal et al.[22] suggested that animals with the A2A2 genotype had the highest milk yield and protein yield compared to alternative variants. Olenski et al.[30] reported a significant association between the A1 allele and lower levels of milk yield traits. Moreover, Heck et al.[31] observed that cows with the A1 allele had a lower protein yield than cows with the A2 allele, resulting from decreased milk production. The positive effects of the A2 variant on milk production traits have also been reported by Morris et al.[32] and Nilsen et al.[33] On the contrary, Kucerova et al.[34] found that the genotype A1A1 was associated with the highest breeding value for milk yield in Czech Fleckvieh cattle. It is important to note that an increase in milk yield negatively affects dairy cow fertility.[35] Ardicli et al.[19] pointed out a possible undesirable association of the A2A2 genotype with some reproduction traits in Holstein-Friesian cows. These researchers observed that animals with the A2A2 genotype had higher means for days before first insemination and first insemination to pregnancy interval. It is important to note that the genomic testing results revealed that the A2A2 genotype had the highest TPI and NM$ values. Table 4 shows the means and standard deviations of TPI and NM$ values of animals with A1A1, A1A2, and A2A2 genotypes. The A2A2 animals have significantly more outstanding genetic merit ($p < 0.001$) than heterozygotes and those with the A1A1 genotype (Fig. S2). However, in the present study, the superiority of the A2A2 genotype was not substantiated concerning milk yield traits. In previous studies, on the other hand, there are remarkable differences in population size, breed, genotype frequencies, the effects of different genetic variants, the power of statistical models, and methods of expressing traits that may affect the results. The cows' characteristics should also be

Table 4. The means and standard deviations for genetic merit of animals with A1A1, A1A2, and A2A2 genotypes in the entire Holstein-Friesian population ($n = 1200$).

	CSN2 genotypes			
	A1A1	A1A2	A2A2	Significance
TPI	2321.60[b] ± 166.90	2358.12[b] ± 169.17	2401.61[a] ± 161.35	$p < 0.001$
NM$	370.80[b] ± 169.41	392.14[b] ± 180.12	441.64[a] ± 161.02	$p < 0.001$

TPI: type production index; NM$: net merit.
[a,b]Means with different superscripts indicate statistical significance.

considered, including age, health status, lactation stage, and parity.[22,36] Detailed studies with appropriate experimental designs in large populations are required before drawing reliable conclusions about the effect of the A1/A2 allele difference on milk yield traits.

Genomic testing provides better estimates of the genetic merit of animals and can be performed at a young age. Genomic test reports consist of PTAs for several individual traits, from milk yield to DPR. The data also contains indices that combine multiple traits weighted by their relative importance, called TPI and NM$.[37] These indices are very convenient for statistical analysis because they are highly processed data in which most non-genetic influences are excluded and represent the relative advantage in milk performance traits.[38] After evaluating the effects of *CSN2* A1/A2 genotypes on milk yield, we examined whether any difference occurred in cows with high and relatively low genetic merit. In this context, we used the PTA values for milk, fat, protein, and DPR as data for ANOVA and evaluated the effects of *CSN2* genotype × genetic merit on phenotypic traits through interaction analysis. Genomic testing is widely used in dairy cattle. The data obtained from these tests allow an accurate selection. Farms want to add genetically superior animals to the herd to receive calves with higher genetic merit. Therefore, it is essential to specifically examine the effects of *CSN2* genotypes in animals with above and below-average genetic merit. As shown in Table 3, we found no statistically significant difference in the *CSN2* genotype × genetic merit interaction analysis. Concerning the EcoFeed® results, the mean values were also highly similar across the three distinct genotypes, and no significant statistical variances were observed (Fig. S3). We observed no statistically significant outcomes upon incorporating the genomic prediction values (milk yield, fat, protein, fat%, protein%, and DPR) into the model as covariates for quantifying lactation milk yield (Fig. S4).

A2 milk production is a promising application for dairy businesses. If the adverse effects of A1 milk on human health become more confident with reliable experimental research, A2 milk will find a much larger market. On the other hand, Kearney et al.[39] demonstrated that sire selection decisions by commercial dairy farmers are substantially more complicated when information is available for specific genetic loci. Therefore, findings on the impact of beta-casein genotypes at the herd level are precious. This study provides very detailed data on the subject. However, it would be helpful to evaluate larger phenotypic datasets, including milk content and reproductive parameters.

Conclusions

This paper presents the genotypic distribution, population genetics, and diversity parameters in a large and genomically tested Holstein-Friesian cow population. It also reports the effects of the *CSN2* genotypes on lactation milk yield and test-day milk yield. In the total population, we observed a remarkably high frequency of the A2 allele. Study data showed a considerable frequency of heterozygotes in the herd. The marker showed mild variability and diversity. The A2A2 cows had remarkably higher genomic merit compared to alternative variants. Although the heterozygote cows produced higher milk yield than homozygote A1 and A2 genotype carriers, the *CSN2* genotypes did not significantly affect milk yield traits in the statistical analysis. A similar finding is valid for the interaction analysis of the *CSN2* genotypes × genetic merit regarding the genomic test results. The selection rate for the A2 variant of the bovine *CSN2* gene and the unintentional use of bulls with the A2A2 genotype has gradually increased in dairy cattle. It is worth noting that the phenotypic effects of the *CSN2* marker should be monitored. We think the data presented here may be useful for further investigations and applications on A2 milk production. A2 herd conversion is a complex issue. Phenotype/genotype association screening supported by widespread genomic testing is needed not only to provide a robust selection program but also to increase the profit of dairy farms.

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Author contributions

Sena Ardicli: genetics methodology, conceptualization, laboratory experiments, statistical analysis, writing the manuscript, review, and editing. Ozgur Aldevir: data collection, animal management, laboratory experiments. Emrah Aksu: data collection, animal management. Ahmet Gumen: methodology, conceptualization and supervision, review, and editing.

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Data availability statement

The data used to support the findings of this study are included in the paper.

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